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# The Protective Effect of Rivaroxaban and Dabigatran on Cardiovascular Complications in Albino Rats with Metabolic Syndrome

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## Abstract

Metabolic syndrome (MetS) represents a combination of diabetes, hypertension, obesity, and other co-morbidities, which puts a greater risk of developing coronary heart disease, stroke, and other vascular complications as thromboembolic events. The role of this study is to evaluate the potential cardiovascular protective effect of rivaroxaban and dabigatran on metabolic syndrome in albino rats. The experimental design consists of a pretreatment phase (0-5$^{th}$ weeks) and a treatment phase (6$^{th}$-8$^{th}$ weeks). Forty adult male albino rats were randomly divided into 4 groups 10 rats each. Group 1: rats were fed a standard chow diet and received normal saline (1 ml) in the treatment phase. During the pretreatment phase, the other 30 rats were fed a high-fructose diet and fructose drinking water for 5 weeks to induce metabolic syndrome. Group 2, MetS group, received normal saline (1 ml); Group 3, MetS group treated with rivaroxaban at a dose of 3 mg/kg/day; and Group 4, MetS group treated with dabigatran at a dose of 15 mg/kg/day. MetS groups treated with rivaroxaban or dabigatran showed a significant improvement in mean body weight and abdominal circumference, a reduction in QRS and QTc intervals, and an improvement in systolic blood pressure. There was also an appreciated refinement in different biochemical parameters and a remarkable improvement in histopathological and immunohistochemical findings. Rivaroxaban and dabigatran revealed potent protective cardiovascular effects on metabolic syndrome and diabetes mellitus.

**Keywords:** Metabolic syndrome, Cardiovascular, Rivaroxaban, Dabigatran, Diabetes mellitus, insulin resistance

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## 1. Introduction

Metabolic syndrome (MetS) is a combination of metabolic disorders including hypertension, insulin resistance, dyslipidemia, abdominal obesity, hyperglycemia, hyperuricemia, and endothelial dysfunction [1], [4]. It is associated with numerous co-morbidities, including increased risk for cardiovascular diseases, dementia, obstructive sleep apnea syndrome, non-alcoholic fatty liver disease, reproductive disorders, and certain forms of cancer [2]. The mechanism of developing type II diabetes mellitus (DM) is thought to be by hindering the regulatory effect of insulin on metabolism. Patients also show obesity associated with decreased insulin sensitivity and beta-cell compensatory mechanisms, such as hyperinsulinemia and increased basal insulin secretion. Therefore, MetS and DM are related metabolic conditions that, on several occasions, may co-exist [3]. DM presents specific changes resulting in hypercoagulable and prothrombotic status. It is mainly due to platelet dysfunction caused by the presence of several factors such as obesity, hyperglycemia, dyslipidemia, insulin deficiency or resistance, inflammation, and cellular abnormalities such as increased platelet turnover, enhanced production of thrombin, and reduced generation of nitric oxide (NO) [5]. Vascular injury that occurs with atherosclerotic disease is characterized by the production and release of inflammatory cytokines and reactive oxygen species (ROS) generation, causing endothelial dysfunction. Among the proinflammatory cytokines, tumor necrosis factor-α (TNF-α) is a cornerstone in keeping systemic inflammation at low levels [6]. For the prevention of either primary or secondary thromboembolic events, antithrombotic therapies could be used, thus attenuating the influence of thrombosis on the overall diabetes effect [7].

Rivaroxaban belongs to the group of direct oral anticoagulants that exert their anti-coagulant activity by inhibiting factor Xa. It is indicated for the prevention of atherosclerotic complications after acute coronary syndrome, venous thromboembolic events, stroke, and systemic embolism, as well as the treatment of deep vein thrombosis and pulmonary embolism [8-9]. Dabigatran is an oral anticoagulant that suppresses thrombin directly and plays an important role in patients with atrial fibrillation for the prevention of thrombotic complications [10]. Furthermore, research findings indicate that dabigatran prevented the development of atherosclerosis in a model of hypercholesterolemia in mice [9], [11-12]. The findings of these studies suggested a connection between vascular protection and dabigatran's inhibition of thrombin [13]. The aim of the current study is to investigate the possible protective effects of rivaroxaban and dabigatran on cardiovascular complications in MetS-induced albino rats.

## 2. Materials and Methods

### *2.1. Drugs and chemicals*

Rivaroxaban (Rivarospire 2.5mg ATCO Pharmaceuticals, for Inspire Pharmaceuticals Company, Cairo, Egypt), Dabigatran (Pradaxa 110mg Boehringer Ingelheim, Cairo, Egypt), and Fructose (Unifructose powder Unipharm, Cairo, Egypt) were used in the current study.

### *2.2. Animals*

Forty adult male albino rats weighing (160-180 gm) were used. They were obtained from the animal house and facilities of of El-Nahda University, Beni-Suef, Egypt. Before being used in lab tests, the animals were housed for 14 days to allow them to acclimate. All drug administration and animal handling experiments were authorized by the Institutional Animal Care and Use Committee, Beni-Suef University (BSU-IACUC) (approval No.021-161, revised 2020), which complies with the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals. The animals were housed in individual cages with a 12-hour light-dark cycle and a regulated temperature of (26±2$^{o}$ C). Ad libitum food and water were provided.

### *2.3. Instruments*

A glucometer (Bionime One Touch; Blood Glucose Monitoring System, Shanghai, China), a blood pressure measurement system (Rat Tail NIBP System; AD Instruments Pty Ltd., Sydney, Australia), an ECG recording system (Power Lab system AD Instruments Pty Ltd., Sydney, Australia) and a digital scale for weight measurement were used in the current study.

### *2.4. Methods*

The present study was performed over 8 weeks. The experimental design consists of a pretreatment phase (0-5$^{th}$ weeks) and a treatment phase (6$^{th}$-8$^{th}$ weeks). Animals were randomly allocated to four groups; each group included 10 rats.

#### *2.4.1. Normal control group*

Rats were fed a standard chow diet composed of 52.8% carbohydrates, 9.2% fat, and 38% protein [14] and tap water for 8 weeks. They received vehicle (Normal saline, 1 ml) in the treatment phase (6$^{th}$-8$^{th}$ weeks). During the pretreatment phase, the other 30 rats will be fed a high-fructose diet (HFD) and fructose drinking water for 5 weeks to induce metabolic syndrome. The HFD is composed of fructose mixed with a normal chow diet in a ratio of 6:4 [15-16]. Every other day, fresh fructose drinking water was made. Fructose (20 grams) was diluted with 100 milliliters of tap water to create fructose (20%). To stop fermentation, aluminum foil was placed over the bottles. For five weeks, fructose-feeding water was given as needed each day [1]. At the end of the 5$^{th}$ week, HFD-fed rats were randomly allocated to one of the following treatment groups: MetS group, rats received vehicle (normal saline, 1 ml). In the MetS group treated with rivaroxaban, rats received oral rivaroxaban (3 mg/kg/day) for 21 days [17] and in the MetS group treated with dabigatran, rats received oral dabigatran (15mg/kg/day) for 21 days [18]. During the treatment phase (6$^{th}$-8$^{th}$ weeks), the 3 treatment groups continued to be fed HFD and fructose drinking water (Figure 1).

### *2.5. Measurements*

#### *2.5.1. Anthropometric measurements*

The body weight and abdominal circumference of each rat were measured at the start, after 5 weeks, and at the end of the experiment. Heart weight (absolute and relative) was recorded at the end of the 8th week of the experiment before being processed for histological examination.

#### *2.5.2. Electrocardiography (ECG)*

ECG recordings and measurement of heart rate were obtained simultaneously after anesthetizing rats by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) [19]. Each rat was anesthetized, then three needle electrodes were connected to the rat's body in a specific order. A Labchart system was used to record ECG data, including HR beat per minute (bpm), RR interval milliseconds (ms), PR interval (ms), P duration (ms), QRS interval (ms), and QT Interval (ms).

#### *2.5.3. The Measurement of systolic blood pressure*

(SBP) was monitored with a manometer-tachometer that used an inflatable tail-cuff and was connected to a Power Lab data-collecting device and an MLT844 physiological pressure transducer. Rats were put in a plastic holder that was fixed on a warm plate with a thermostat, which kept the temperature at 35 °C while the measurements were being taken. One week prior to the trial, all animals were prepared for SBP measurements. The tail cuff is inflated to obscure the blood flow until arterial pressure disappears, and when the pressure wave first appears during deflation, it is recorded as the systolic pressure. After the animals were accustomed to their surroundings, an average value from three SBP readings

(that varied by no more than 2 mm Hg) was obtained for each rat [2].

### *2.5.4. Biochemical assays*

#### *Collection of serum blood samples*

Retro-orbital sampling was done in the 8th week under inhalation anesthesia through alcohol, chloroform, and ether in a ratio of 1:2:3 [20]. After obtaining a sample volume of up to 3 milliliters, the drawn blood was centrifuged for 20 minutes at a rate of 3000 rounds per minute. After that, the serum was put into sterile vials and kept in a freezer at -20°C for biochemical parameters assessment. The serum of the various rat groups was assessed for the following biochemical parameters:

***1. Lipid profile*** includes triglycerides (TG)**,** total cholesterol (TC)**,** low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C).

***2. Cardiac enzymes*** including lactic acid dehydrogenase (LDH)**,** creatine kinase-myocardial band (CK-MB) and troponin I [21].

***3. Insulin resistance index***

**HOMA-IR=** fasting blood glucose (mmol/L) x fasting insulin (μIU/ mL) [24]

***4. Oxidant antioxidant:*** myeloperoxidase (MPO) [26], malondialdehyde (MDA) [27], and glutathione (GSH) [22].

***5. Coagulation tests:*** activated partial thromboplastin time (APTT), and prothrombin time (PT) [17].

### *2.5.5. Histopathological examination of the heart and aorta*

Following blood samples collection at the final stage of the study, all animals were sacrificed. Rats from various groups had their thoracic cavities opened, and their hearts and proximal aortas were collected and prepared for histopathological studies. The specimens were processed utilizing the tissue-embedding facility after fixation in 10% formaldehyde for light microscopic investigation. (LEICA EG 1160, WETZLAR, Germany). The specimens were stained with hematoxylin and eosin (H&E) [25].

### *2.5.6. Immunohistochemically staining for the expression of TNF α of the heart and aortic tissues*

It was performed on the prepared formalin-fixed paraffin-embedded tissues using the primary antibody TNF-α from Biocyc GmbH and CoKG, Luckenwalde, Germany, and staining was performed using the Dako Autostainer Link 48 (Agilent Technologies, Santa Clara, CA).

### *2.6. Statistical analysis*

Results were presented as mean ± standard deviation (SD) and were analyzed for statistically significant differences using non-parametric Kruskal–Wallis one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis to compare the means from different groups. A P-value < 0.05 was considered significant. SPSS 26 for Windows was used for statistical calculations (San Diego, CA, USA).

## 3. Results and discussion

In figure 1 All drugs were given daily orally during the last 3 weeks of HFD feeding through a metallic tube dissolved in normal saline.

### *3.1. Anthropometric Measurements*

#### *3.1.1. Body weight (BW), abdominal circumference (AC) and Heart weight (HW)*

The results of the current study revealed that rivaroxaban and dabigatran treatment alleviated significantly both the increased BW and AC induced by MetS in 5th and 8th week with no significant effect on both increased absolute and relative HW induced by MetS (table 1). A hypercoagulable state has been observed in metabolic syndrome, which may be caused by endothelial dysfunction, elevated coagulation factor activity, platelet hyperactivity, and reduced fibrinolysis. Antithrombotic medications may therefore be useful in preventing thromboembolic events in both primary and secondary settings, hence reducing the effect of thrombosis associated with diabetes Mellitus [7], [4]. In the current study, a high-fructose diet (HFD) and fructose drinking water induced MetS in experimental rats that is in agreement with many previous studies that showed that a high fructose diet induced MetS [28-30]. MetS was evident in the present study by a statistically significant rise in mean body weight, abdominal circumference and increased TG, TC, and LDL levels with a significant reduction in HDL levels, a significant elevation of fasting blood glucose, insulin, HOMA-IR ratio, CK-MB, Troponin I, LDH, MDA and MPO levels and a decreased GSH level, a statistically significant reduction of PT and APTT with elevated HR and SBP. Regarding body weight and abdominal circumference, MetS groups treated with rivaroxaban or dabigatran showed a statistically significant alleviation in mean body weight and abdominal circumference when compared to the MetS group i.e., the tested drugs could not normalize the body weight while they reduced the effect of HFD on the body weight. The present results correlate with Kopec et al., (2017) who revealed that treatment with dabigatran reduced the development of HFD-induced obesity and attenuated the development of obesity-related consequences in mice with obesity. These results can be clarified as fibrinogen and thrombin activity could exacerbate diet-induced obesity, metabolic inflammation, and associated consequences through several mechanisms [31]. Fibrinogen changes the function of adipocytes or preadipocytes by directly engaging them, and through adipose tissue macrophages mediated mechanisms beyond the generation of proinflammatory mediators so targeting thrombin or fibrinogen may reduce pathologies in obese patients as extravascular fibrinogen is a potent proinflammatory disease modifier [31-32]. Regarding heart weight, the three MetS groups presented with a statistically significant increase in absolute and relative heart weight as compared to the normal control group. These results are in

agreement with Park et al., (2020) and Zayed et al., (2018) which can be explained as MetS induce heart remodeling, increase cardiomyocyte size, cardiac interstitial fibrosis and macrophage infiltration [33-34].Regarding ECG and heart rate (HR), MetS rats treated with dabigatran showed a statistically significant prolongation in RR interval and a statistically significant decrease in HR as compared to MetS rats and both rivaroxaban and dabigatran treated rats clarified a statistically significant reduction in QRS and in QTc interval as compared to MetS rats. These results are in harmony with Ring et al., (2013) who stated that therapeutic and fourfold supratherapeutic doses of dabigatran do not cause QT interval prolongation. This can be explained as dabigatran has no effect on cardiac repolarization demonstrating absence of effect on cardiac ion channels [35]. Also, Kubitza et al., (2008) reported that rivaroxaban does not induce QTc interval prolongation. The improvement in heart rate and QTc interval prolongation can be explained by the overall improvement in the criteria of MetS. Regarding systolic blood pressure, MetS rats treated with rivaroxaban or dabigatran clarified a statistically significant reduction in SBP as compared to MetS group [36]. These results agree with Namba et al., (2017) revealed that rivaroxaban decreased arterial stiffness and improved atherosclerosis [37]. Also, Barrios and Escobar, (2013) showed that dabigatran improved blood pressure control in atrial fibrillation [38]. This can be explained as the suppression of thrombin activity by dabigatran enhances the vascular endothelium NO-dependent function and normalizes the 20-hydroxyeicosatetraenoic dependent pathway [39]. On the contrary, Ware et al., (2015) showed that in sham-operated rats, treatment with dabigatran revealed a dose-dependent elevation in systolic BP only, but this was only evident with dabigatran doses of 50 mg/kg/day or more [40]. Also, Kubitza et al., (2013) showed that rivaroxaban has no effect on blood pressure. Regarding lipid profile, MetS group treated with rivaroxaban exhibited a statistically significant alleviation in TG, TC, and LDL and a significant increase in HDL levels when compared to the MetS group [41-44]. The mechanism can be attributed to key clotting proteases, such as factor Xa (FXa), that can induce atherosclerosis, via protease-activated receptors (PARs) so FXa suppression by rivaroxaban reduced the onset of atherosclerosis [45]. However, Zhou et al., (2011) showed that rivaroxaban did not cause a significant difference in TC, LDL-cholesterol, HDL-cholesterol, and TG levels in Apolipoprotein (Apo) E-Deficient Mice [46]. Moreover, the MetS group treated with dabigatran demonstrated a statistically significant alleviation in TG, TC, and LDL and a significant increase in HDL levels when compared to the MetS group [47]. This can be clarified by the dose-dependent effect of dabigatran as it causes a reduction in apolipoprotein B concentration that acts as the primary apolipoprotein of chylomicrons, VLDL, IDL, and LDL particles [48-49]. However, Scridon et al., (2019) stated that dabigatran administration had no effect on lipid profile in both control and diabetic rats [50]. Regarding the fasting glucose, insulin level and insulin resistance, the MetS group treated with rivaroxaban or dabigatran showed a statistically significant alleviation in fasting blood glucose, insulin and HOMA-IR index when compared to MetS group [51-52]. The result may be due to the decrease in insulin resistance because of the reduction in body weight and abdominal circumference. As fibrinogen and thrombin activity exacerbate diet-induced obesity so targeting thrombin or fibrinogen by rivaroxaban or dabigatran may limit pathologies in obese patients [31-32]. Regarding Creatine kinase Myocardial Band (CK-MB), troponin I and lactic Acid Dehydrogenase (LDH), MetS group treated with rivaroxaban clarified a statistically significant alleviation in CK-MB, Troponin I and LDH when compared to MetS group. These results are in accordance with Imam et al., (2020) and Cavender et al., (2015). This can be explained as rivaroxaban, a direct oral anticoagulant, suppresses factor Xa consequently blocking thrombin formation and reducing thrombus propagation so treatment with rivaroxaban will suppress this thrombotic cascade in a way that decreases MI incidence [43], [7]. Also, rivaroxaban reduced stent thrombosis in MI events due to ischemic imbalance [53]. Another mechanism of action is that rivaroxaban inhibits atherosclerosis through FXa suppression as it can reduce lesion area, M1 macrophages accumulation, and contractile-synthetic phenotypic conversion of vascular smooth muscle cells in atherosclerotic plaques by inhibiting FXa-induced PAR2 [54]. Moreover, MetS group treated with dabigatran displayed a statistically significant alleviation in CK-MB, troponin I and LDH when compared to MetS group [55-56]. This can be due to the direct thrombin suppression effect of dabigatran that enhances endothelial function and lowers the amount of collagen, the size of atherosclerotic lesion, and oxidative stress in atherosclerosis with hypercholesterolemia [57].

Moreover, thrombin is a key modulator of other cellular signaling pathways via protease-activated receptor (PAR)-mediated signaling activation. Activation of PAR on the wall of the arterial vessel and heart results in atherosclerosis, so thrombin inhibition by dabigatran decreases atherosclerosis [58]. Song et al., (2018) described that dabigatran inhibits no-reflow phenomenon, decrease infarct size, and elevate arterial pressure in acute MI via the reduction of collagen I, inducible nitric oxide synthase, transforming growth factor β1, α smooth muscle actin, and connective tissue growth factor protein expression in rabbits with acute myocardial infarction, as well as through anti-inflammatory and anti-oxidative activities [59]. On the other side, Douxfils et al., (2014) reported that dabigatran is accompanied with a significantly elevated MI risk [60]. This could be due to underdosing as it was associated with increased incidence of cardiovascular events and hospitalization [61]. Regarding comparison of Oxidant antioxidant, MetS group treated with rivaroxaban demonstrated a statistically significant alleviation in MDA and MPO levels and a significant increase in GSH when compared to MetS group. These results agree with Imam et al., (2020) whose results can be clarified as rivaroxaban restore interleukin 17 (IL-17) levels to normal. According to researchers, IL-17 plays a role in the generation of proinflammatory mediators because IL-17A causes the release of inflammatory cytokines and chemokines that recruit T cells, dendritic cells, and neutrophils [62]. Additionally, rivaroxaban treatment dramatically reduces the mRNA expressions of inflammatory molecules since FXa increases the expressions of these molecules [63]. Moreover, MetS group treated with dabigatran presented

with a statistically significant alleviation in MDA and MPO levels and a marked increase in GSH level when compared to MetS group. These results are in accordance with Ellinghaus et al., (2017) and Pingel et al., (2015). This can be explained as thrombin participates in non-hemostatic processes, such as inflammation [64-65]. The anti-inflammatory effects of dabigatran are mainly due to direct thrombin inhibition leading to reduction of plasma-induced transcriptional changes mediated by thrombin-induced PAR-1 activation [64], [66]. Regarding Prothrombin Time (PT) and activated partial thromboplastin time (APTT), MetS group treated with rivaroxaban exhibited a statistically significant prolongation of PT when compared to MetS rats [51], [67]. The prothrombin time may be a more appropriate test compared to APTT when evaluating the magnitude of anticoagulation in patients on rivaroxaban and is more sensitive to rivaroxaban than to dabigatran [67-68]. These results can be explained as rivaroxaban which extends the initiation phase of thrombin generation, decreases the maximum concentration of thrombin produced, and makes the fibrin structure more permeable and liable to fibrinolysis [69]. Moreover, MetS rats treated with dabigatran exhibited a statistically significant prolongation of APTT when compared to normal control, MetS and rivaroxaban treated groups [70-71]. The mechanism of action can be described as dabigatran reversibly binds to the thrombin molecule's active site, preventing thrombin-mediated activation of clotting factors. Moreover, dabigatran can inactivate thrombin even when thrombin is bound to fibrin [72]. Baglin et al., (2012) stated that the APTT demonstrates a curvilinear dose-response to dabigatran with a steep rise at low concentrations. The APTT also shows a curvilinear response to rivaroxaban but is less sensitive to low drug concentrations compared to dabigatran [73]. Furthermore, the PT is insensitive to dabigatran at low concentrations [74], [75] as there is a significant variation in PT reagent sensitivity [75]. In addition, Samuelson et al., (2017) indicated that in the absence of specific tests, APTT is recommended over PT/international normalized ratio (INR) for evaluation of dabigatran, and PT/INR is recommended over APTT for assessing factor Xa inhibitors as rivaroxaban. Regarding histopathological and immunohistochemical examination, MetS rat heart revealed hemorrhage, necrosis, and collection of inflammatory cells as lymphocytes and histiocytosis while the section of MetS rat aorta revealed perivascular infiltration by inflammatory cells and infiltration of intima by lymphocytes and histiocytes with mild elevation which indicates atherosclerosis. Also, The TNF-α positive staining was observed in cardiac cells and perivascular tissue of the aorta [76]. On the other hand, section of the MetS rat heart treated with rivaroxaban revealed no hemorrhage or necrosis with decreased inflammatory cells while section of the MetS rat aorta treated with rivaroxaban showed decreased perivascular and intimal infiltration by inflammatory cells. Also, a positive reaction for TNF-α was detected in a few cardiac cells as well as in the perivascular aortic tissue. These results correlate with Imano et al., (2018) who revealed that rivaroxaban deceased cardiac remodeling due to intermittent hypoxia through the prevention of oxidative stress and fibrosis [77]. Also, Hara et al., (2015) showed that administration of rivaroxaban reduced the progression of atherosclerotic lesion in the aortic arch of Apo E-deficient mice. In addition, Mahmoud et al., (2019) found that rivaroxaban decreased expression of TNF-α in rats with carbon tetrachloride–induced liver fibrosis [78]. Rivaroxaban decreases Nuclear factor kappa B (NF-κB) pathways activation via PAR-2 as FXa by activation of PAR-2 may be involved in tissue fibrosis and tissue remodeling. It also decreases mRNA expression of TNF-α. In contrast, the absence of PAR-2 reduces histological damage and decreases genes expression associated with oxidative stress and fibrosis [77]. Moreover, the section of MetS rat heart treated with dabigatran revealed decreased foamy and inflammatory cells as histiocytes and lymphocytes while the section of MetS rat aorta treated with dabigatran revealed scattered perivascular infiltration with inflammatory cells and decreased intimal infiltration by lymphocytes and foamy cells. Also, positive cytoplasmic reaction for TNF-α was detected in some cells in cardiac myocytes and perivascular tissue of aorta. These results are in accordance with Dong et al., (2017) who showed that dabigatran decreased fibrosis either perivascular or interstitial, and reduced myocardial fibrosis markers expression as collagen I, III and PAR-1 through inhibition of thrombin activity and down-regulation of PAR-1 expression [79]. Van Gorp et al., (2021) reported that dabigatran significantly reduced the progression of atherosclerotic plaque compared with the control group [80]. Also, Durmaz et al., (2022) stated that dabigatran reduced histopathological inflammatory response and decreased TNF-α level in renal tissue after temporary aortic occlusion through inhibition of PARs [81].

### *3.2. Electrocardiography (ECG)*

The present study showed that MetS rats treated with dabigatran showed a statistically significant alleviation of the shortened RR interval as compared to MetS rats. Both rivaroxaban and dabigatran-treated rats showed a statistically significant alleviation of the prolonged QRS and QTc Intervals (table 2 and figure 2), as well as improvement in the increase in the HR as compared to MetS rats (table 2 and figure 3), with no significant effect on PR Interval (ms) and P-Duration (ms).

### *3.3. Systolic blood pressure (SBP)*

Both rivaroxaban and dabigatran-treated rats showed a significant improvement of the increased SBP as compared to MetS rats (table 2 and figure 3).

### *3.4. Biochemical Assays*

#### *3.4.1. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and Low-density lipoprotein (LDL)*

MetS group treated with rivaroxaban or dabigatran showed a statistically significant reduction of the elevated TC, TG and LDL and a significant increase of the decreased HDL level when compared to the MetS group (Table 3 and Figure 4).

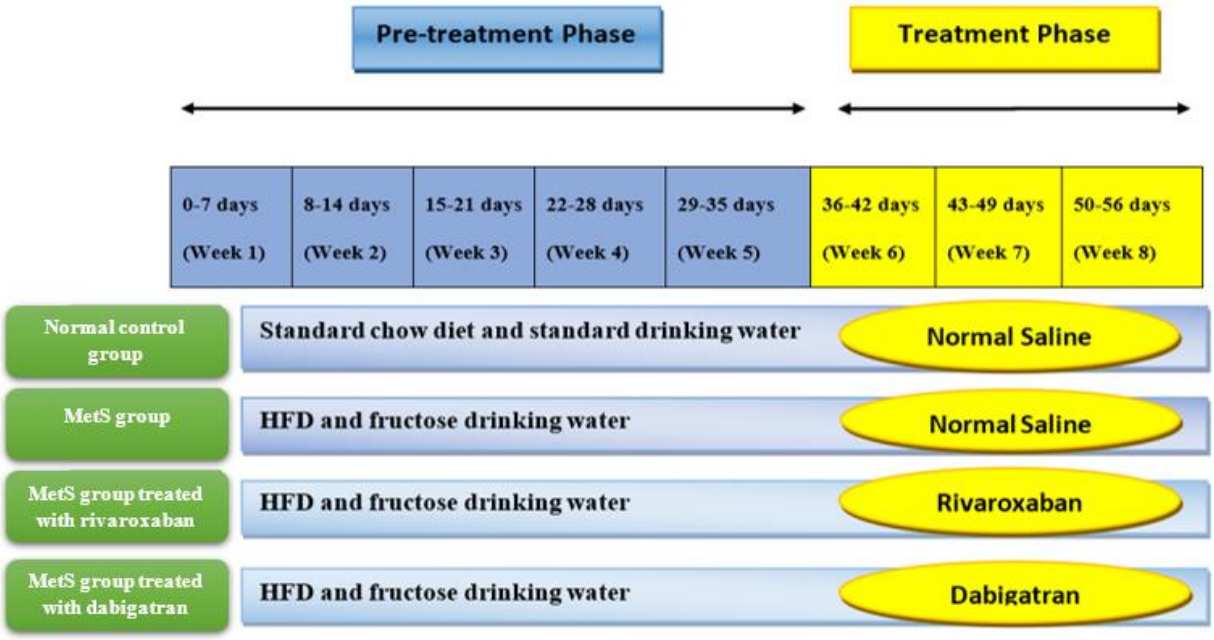



**Figure 1.** Schematic demonstration of the study design

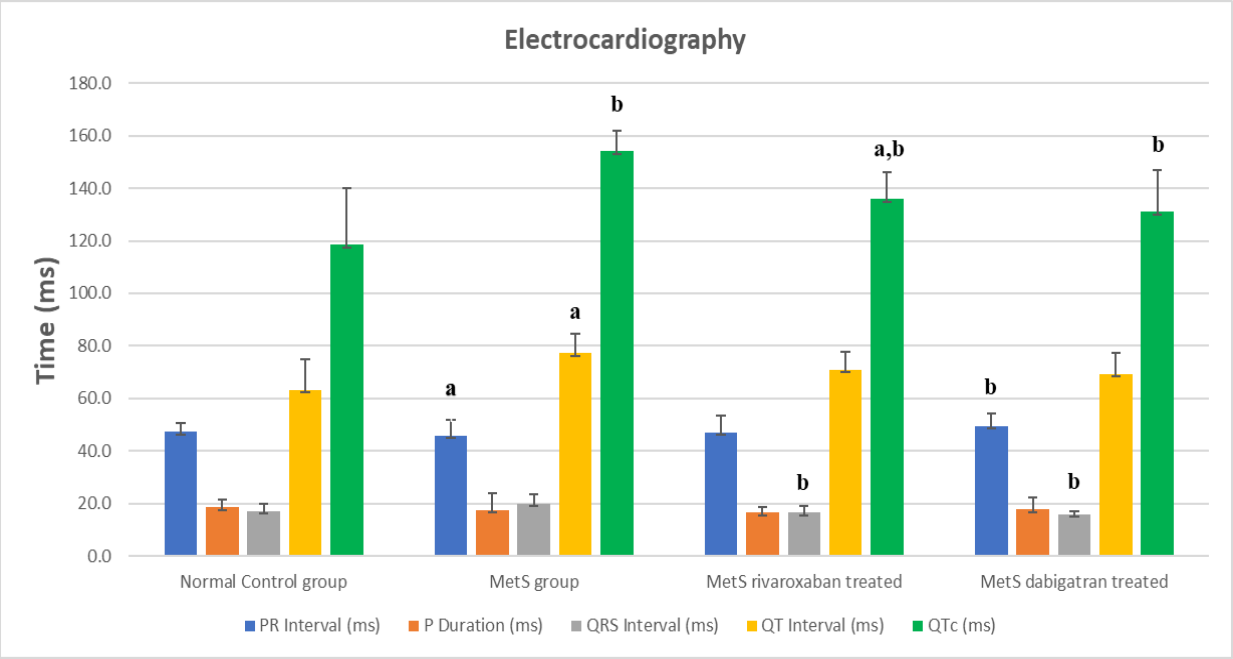



**Figure 2.** The effect of rivaroxaban and dabigatran on ECG in MetS-induced rats

[a] Significantly different from Normal Control Group at $p \leq 0.05$.
[b] Significantly different from MetS Group at $p \leq 0.05$.

**Table 1.** The effect of rivaroxaban and dabigatran on BW, AC and HW in MetS-induced rats

| | | Normal Control N= 6 | MetS N= 8 | MetS rivaroxaban treated. N= 8 | MetS dabigatran treated. N= 8 |
|---|---|---|---|---|---|
| **BW (gm)** | **Baseline** | 165.83 ±5.07 | 164.25 ±5.12 | 166.13 ±7.51 | 168.57 ±8.99 |
| | **5th week** | 190.17 ±9.70 | 258.13 ±17.72[a] | 245.00 ±10.00[a,b] | 252.86 ±4.88[a] |
| | **8th week** | 220.33 ±10.80 | 312.50 ±15.35[a] | 296.88 ±9.23[a,b] | 300.00 ±8.66[a,b] |
| **AC (mm)** | **Baseline** | 14.2 ±0.8 | 14.3 ±0.7 | 13.9 ±1.2 | 14.1 ±1.1 |
| | **5th week** | 16.5 ±0.4 | 18.9 ±0.5[a] | 17.9 ±1.0[a] | 17.8 ±1.1[a] |
| | **8th week** | 18.5 ±0.5 | 22.9 ±1.8[a] | 20.4 ±1.3[a,b] | 20.1 ±1.0[a,b] |
| **Absolute HW (gm)** | **8th week** | 1.55 ±0.19 | 2.31 ±0.28[a] | 2.13 ±0.21[a] | 2.11 ±0.25[a] |
| **Relative HW[#] (mg/gm)** | **8th week** | 83.67 ±8.70 | 100.86 ±9.88[a] | 104.69 ±15.55[a] | 105.51 ±16.27[a] |

[#] relative heart weight was calculated as: RHW= Absolute HW (mg)/BW (gm)
Results are presented as mean ± SD.
Statistical analysis was carried out using non-parametric Kruskal–Wallis one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis.
[a] Significantly different from Normal Control Group at p≤ 0.05.
[b] Significantly different from MetS Group at p≤ 0.05.

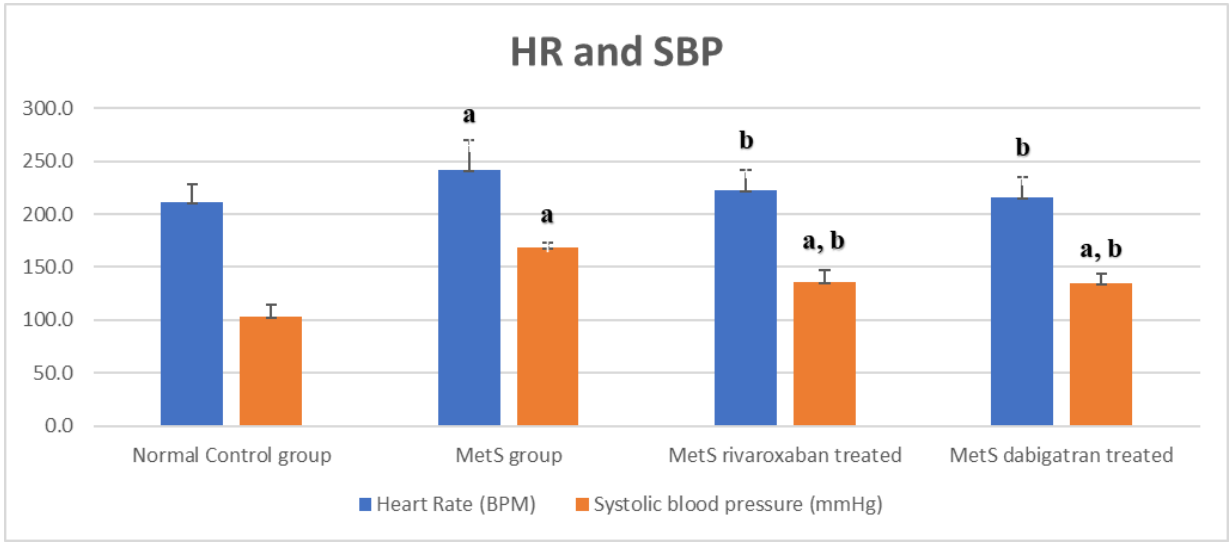



**Figure 3.** The effect of rivaroxaban and dabigatran on HR (bpm) and SBP (mmHg) in MetS-induced rats

[a] Significantly different from Normal Control Group at p≤ 0.05.
[b] Significantly different from MetS Group at p≤ 0.05.

**Table 2.** The effect of rivaroxaban and dabigatran on ECG, HR and SBP in MetS-induced rats

| | **Normal Control N= 6** | **MetS N= 8** | **MetS rivaroxaban treated. N= 8** | **MetS dabigatran treated. N= 8** |
|---|---|---|---|---|
| **RR Interval (ms)** | 285.20 ±22.96 | 252.04 ±32.55[a] | 271.60 ±23.25 | 280.26 ±25.16[b] |
| **PR Interval (ms)** | 47.16 ±3.44 | 45.88 ±6.06 | 46.99 ±6.30 | 49.46 ±4.69 |
| **P Duration (ms)** | 18.50 ±2.84 | 17.39 ±6.41 | 16.47 ±2.32 | 17.62 ±4.54 |
| **QRS Interval (ms)** | 17.09 ±2.54 | 20.02 ±3.65 | 16.43 ±2.56[b] | 15.94 ±1.24[b] |
| **QT Interval (ms)** | 63.25 ±11.54 | 77.28 ±7.30[a] | 70.87 ±6.67 | 69.25 ±8.12 |
| **QTc (ms)** | 118.54 ±21.59 | 154.06 ±7.88[a] | 135.96 ±9.98[a,b] | 131.03 ±15.85[b] |
| **HR (BPM)** | 211.53 ±16.85 | 241.26 ±28.32[a] | 222.39 ±18.91[b] | 215.61 ±19.23[b] |
| **Systolic blood pressure (mmHg)** | 103.50 ±11.15 | 168.21 ±4.73[a] | 135.99 ±10.75[a,b] | 134.69 ±9.07[a,b] |

Results are presented as mean ± SD.
Statistical analysis was carried out using non-parametric Kruskal–Wallis one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis.
[a] Significantly different from Normal Control Group at $p \leq 0.05$.
[b] Significantly different from MetS Group at $p \leq 0.05$.

**Table 3.** The effect of rivaroxaban and dabigatran on biochemical profile in MetS-induced rats

| | **Normal Control N= 6** | **MetS N= 8** | **MetS rivaroxaban treated N= 8** | **MetS dabigatran treated N= 8** |
|---|---|---|---|---|
| **TG** (mg/dl) | 99.00 ±12.94 | 187.40 ±23.43[a] | 127.80 ±5.45[a,b] | 127.00 ±12.04[a,b] |
| **TC** (mg/dl) | 142.00±11.20 | 256.00 ±43.28[a] | 167.80 ±8.23[b] | 174.20 ±16.81[b] |
| **LDL** (mg/dl) | 65.17 ±5.61 | 126.10 ±6.96[a] | 78.85 ±5.40[a,b] | 76.97 ±5.86[a,b] |
| **HDL** (mg/dl) | 67.60 ±8.76 | 27.60 ±5.41[a] | 41.80 ±3.83[a,b] | 44.40 ±4.77[a,b] |
| **Fasting Blood Glucose (mmol/L)** | 5.54 ±0.44 | 16.12 ±2.44[a] | 9.62 ±0.86[a,b] | 9.70 ±0.61[a,b] |
| **Insulin (mU/ml)** | 8.90 ±0.71 | 21.24 ±2.26[a] | 14.92 ±1.83[a,b] | 13.40 ±1.54[a,b] |
| **HOMA-IR ratio** | 2.18 ±0.13 | 15.20 ±2.59[a] | 6.38 ±0.94[a,b] | 5.80 ±0.90[a,b] |
| **CKMB (U/ml)** | 119.82±6.18 | 227.02 ±18.46[a] | 144.86 ±12.00[a,b] | 140.98 ±14.53[a,b] |
| **Troponin-I(Pg/ml)** | 7.16 ±0.44 | 13.62 ±1.40[a] | 9.50 ±0.52[a,b] | 9.36 ±0.62[a,b] |
| **LDH (U/ml)** | 93.10 ±8.01 | 197.28±10.98[a] | 112.64 ±7.72[a,b] | 109.96 ±8.36[a,b] |
| **MDA (nmol/ml)** | 30.46 ±6.7 | 126.20 ±10.11[a] | 74.82 ±11.02[a,b] | 82.74 ±6.16[a,b] |
| **GSH (mmol/ml)** | 225.12 ±14.71 | 116.94 ±9.38[a] | 186.84 ±19.54[a,b] | 186.46 ±14.78[a,b] |
| **MPO (mmol/ml)** | 7.30 ±0.84 | 26.46 ±7.91[a] | 12.38 ±2.06[b] | 12.08 ±2.10[b] |
| **PT (sec.)** | 26.30 ±5.40 | 18.91 ±3.53[a] | 27.73 ±6.55[b] | 24.00 ±5.67 |
| **APTT (sec.)** | 45.28 ±4.10 | 39.43 ±6.90 | 46.53 ±8.66 | 66.13 ±16.73[a,b,c] |

Results are presented as mean ± SD.
Statistical analysis was carried out using non-parametric Kruskal–Wallis one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis.
[a] Significantly different from Normal Control Group at $p \leq 0.05$.
[b] Significantly different from MetS Group at $p \leq 0.05$.

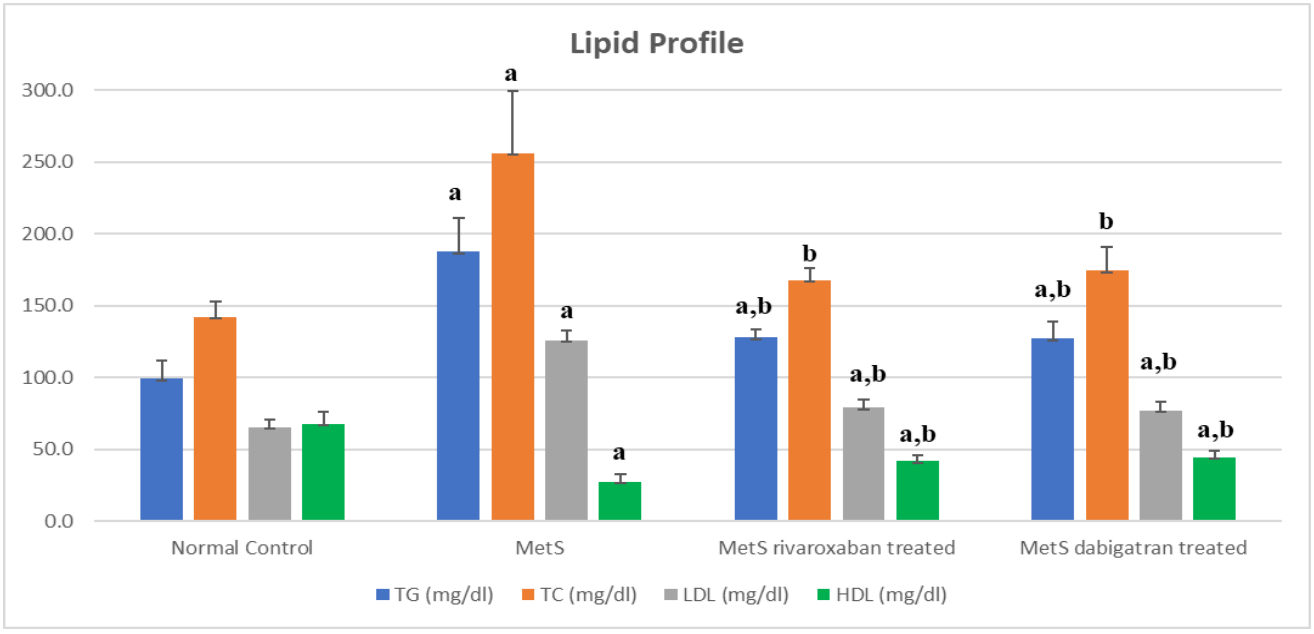



**Figure 4.** The effect of rivaroxaban and dabigatran on TC, TG, HDL and LDL in MetS-induced rats

[a] Significantly different from Normal Control Group at p≤ 0.05.
[b] Significantly different from MetS Group at p≤ 0.05.

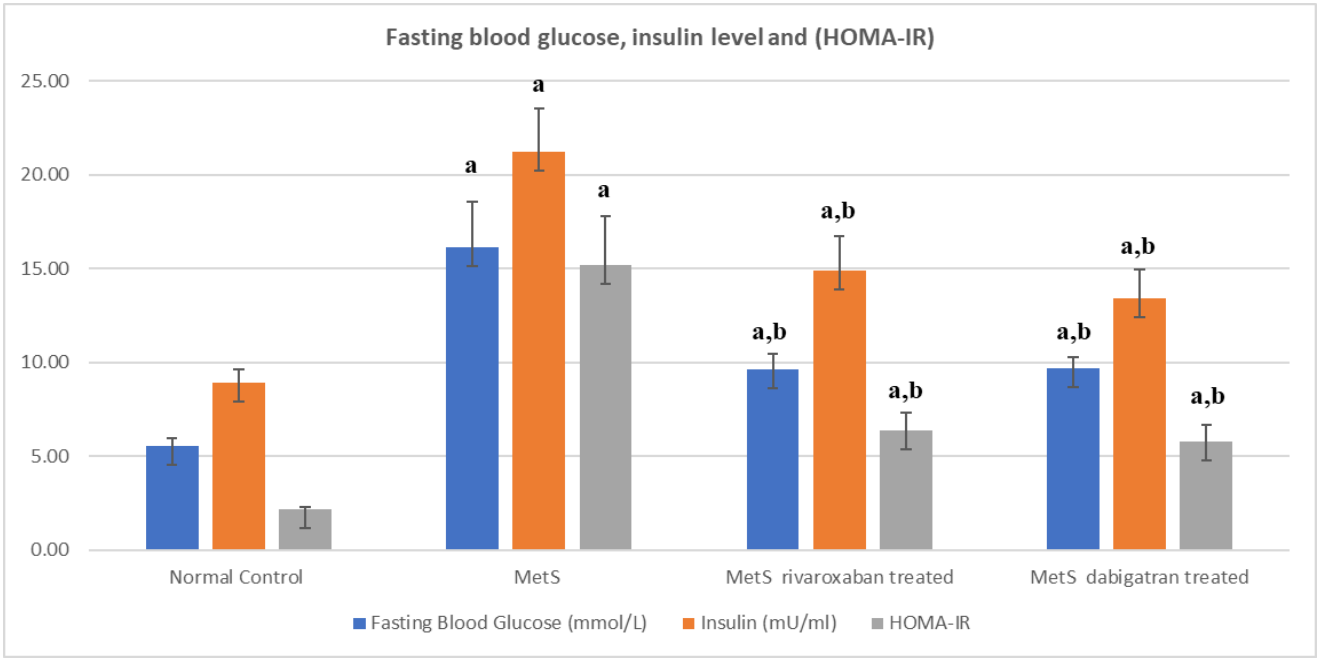



**Figure 5.** The effect of rivaroxaban and dabigatran on fasting blood glucose, insulin level and HOMA-IR in Mets-induced rats

[a] Significantly different from Normal Control Group at p≤ 0.05.
[b] Significantly different from MetS Group at p≤ 0.05.

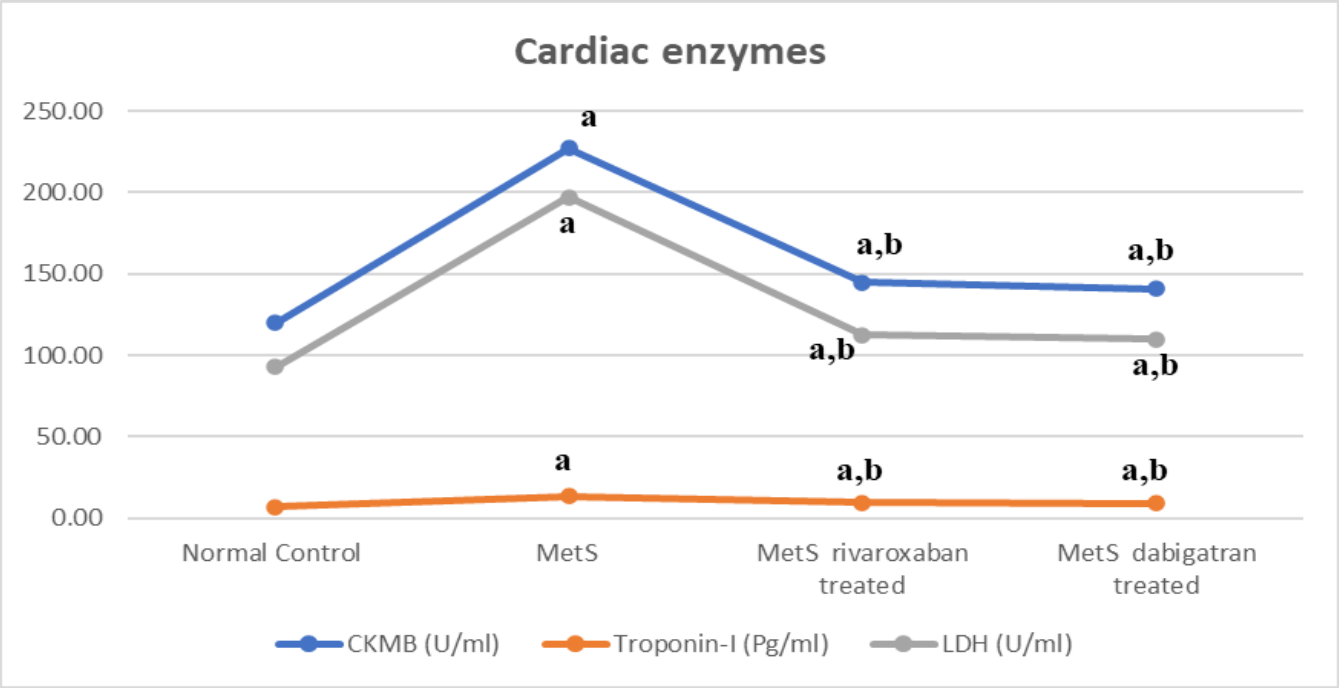



**Figure 6.** The effect of rivaroxaban and dabigatran on CKMB, Troponin I and LDH in Mets-induced rats.

[a] Significantly different from Normal Control Group at p≤ 0.05.
[b] Significantly different from MetS Group at p≤ 0.05.

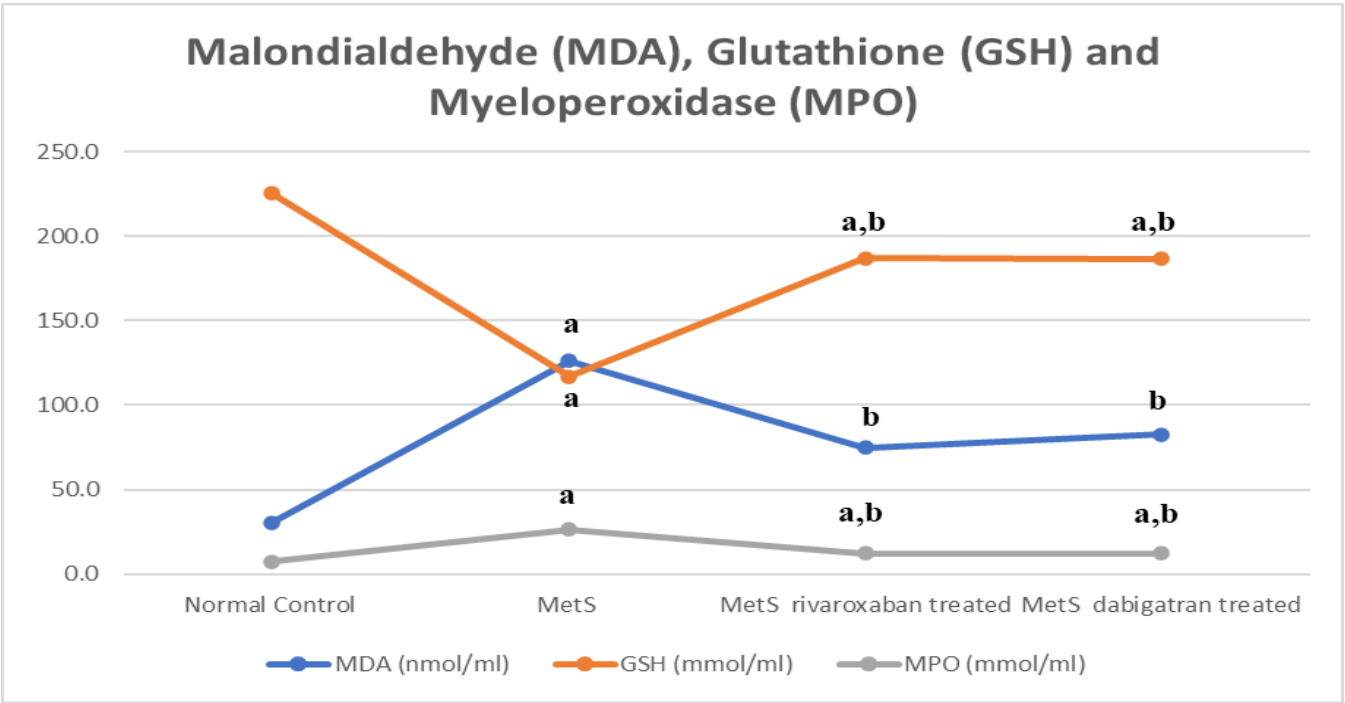



**Figure 7.** The effect of rivaroxaban and dabigatran on MDA, GSH and MPO in Mets-induced rats

[a] Significantly different from Normal Control Group at p≤ 0.05.
[b] Significantly different from MetS Group at p≤ 0.05.

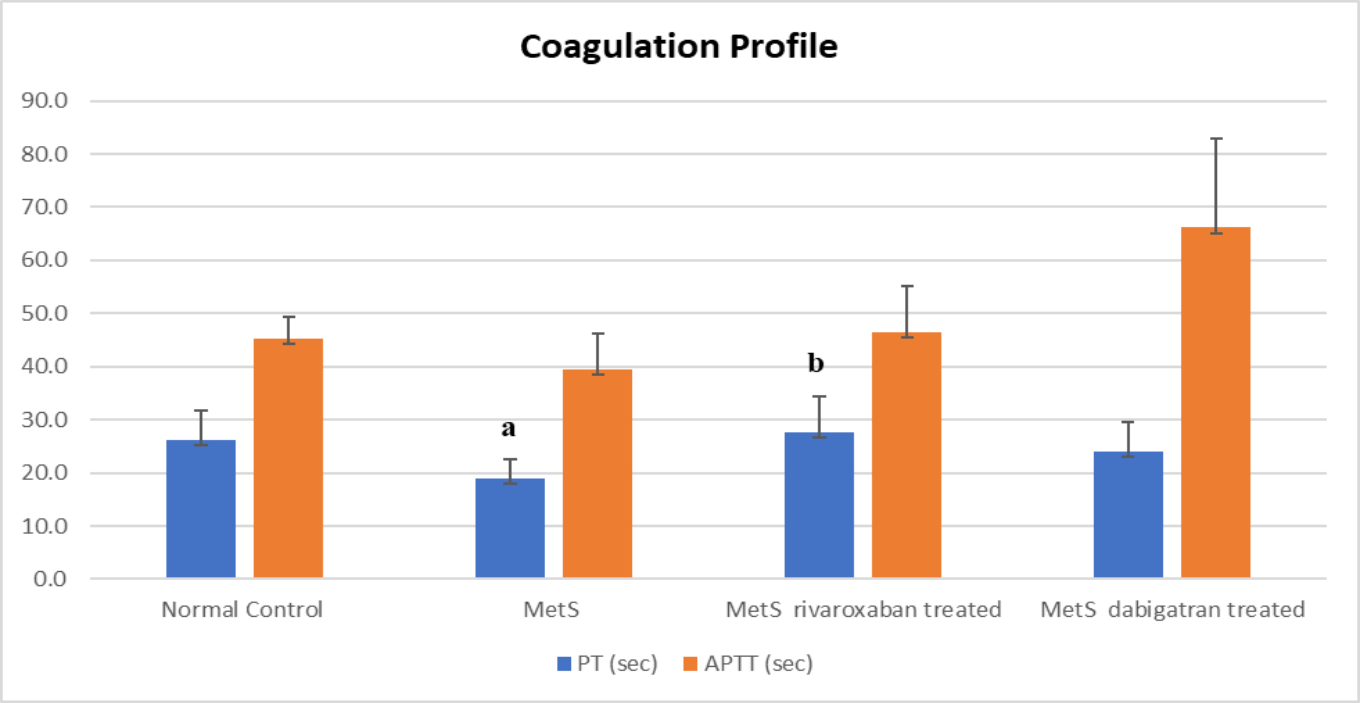
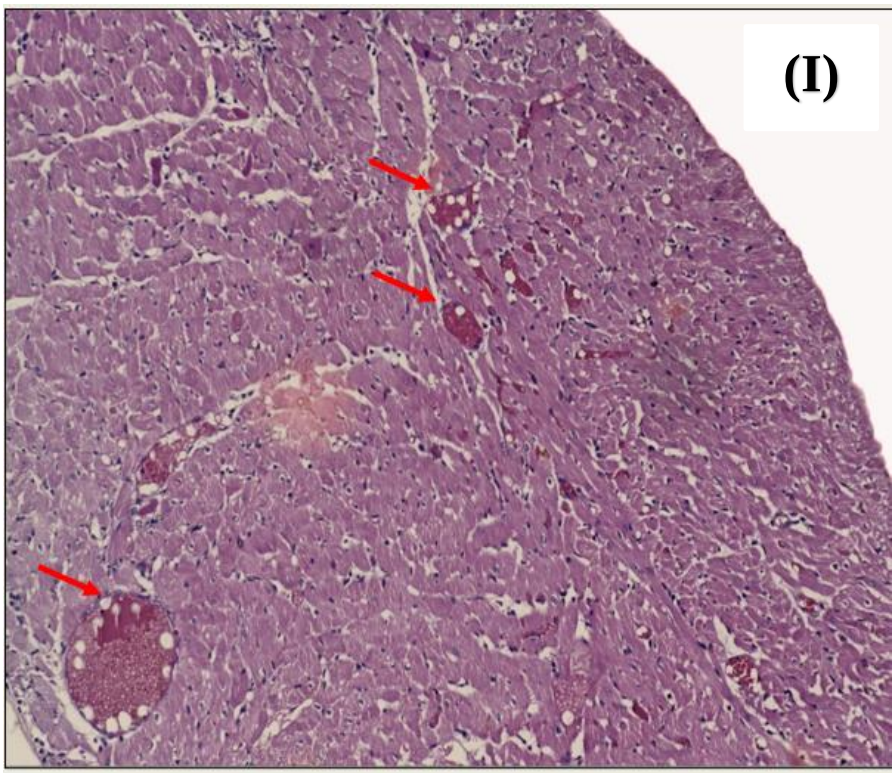
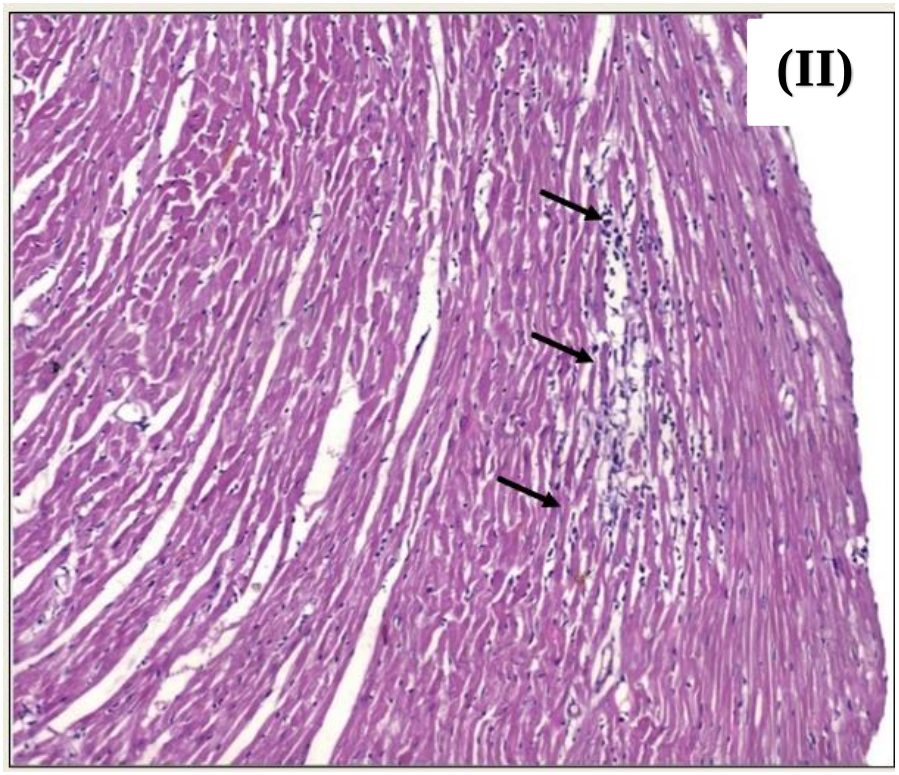



**Figure 8.** The effect of rivaroxaban and dabigatran on PT and APTT in MetS-induced rats

[a] *Significantly different from Normal Control Group at p≤ 0.05.*
[b] *Significantly different from MetS Group at p≤ 0.05.*

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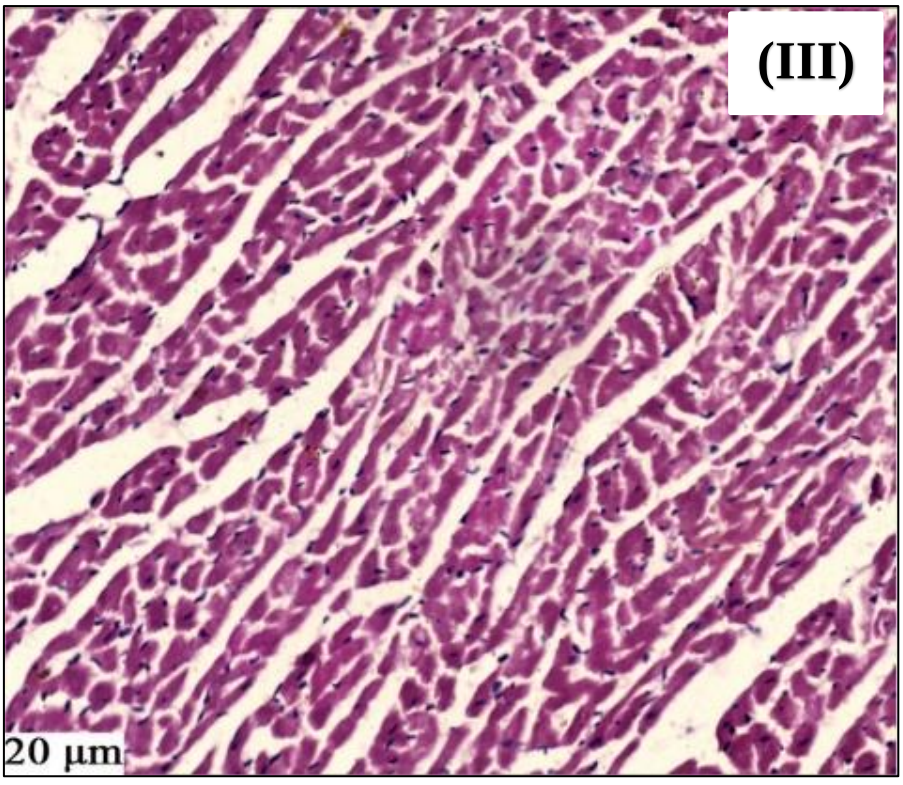

(III)
20 µm

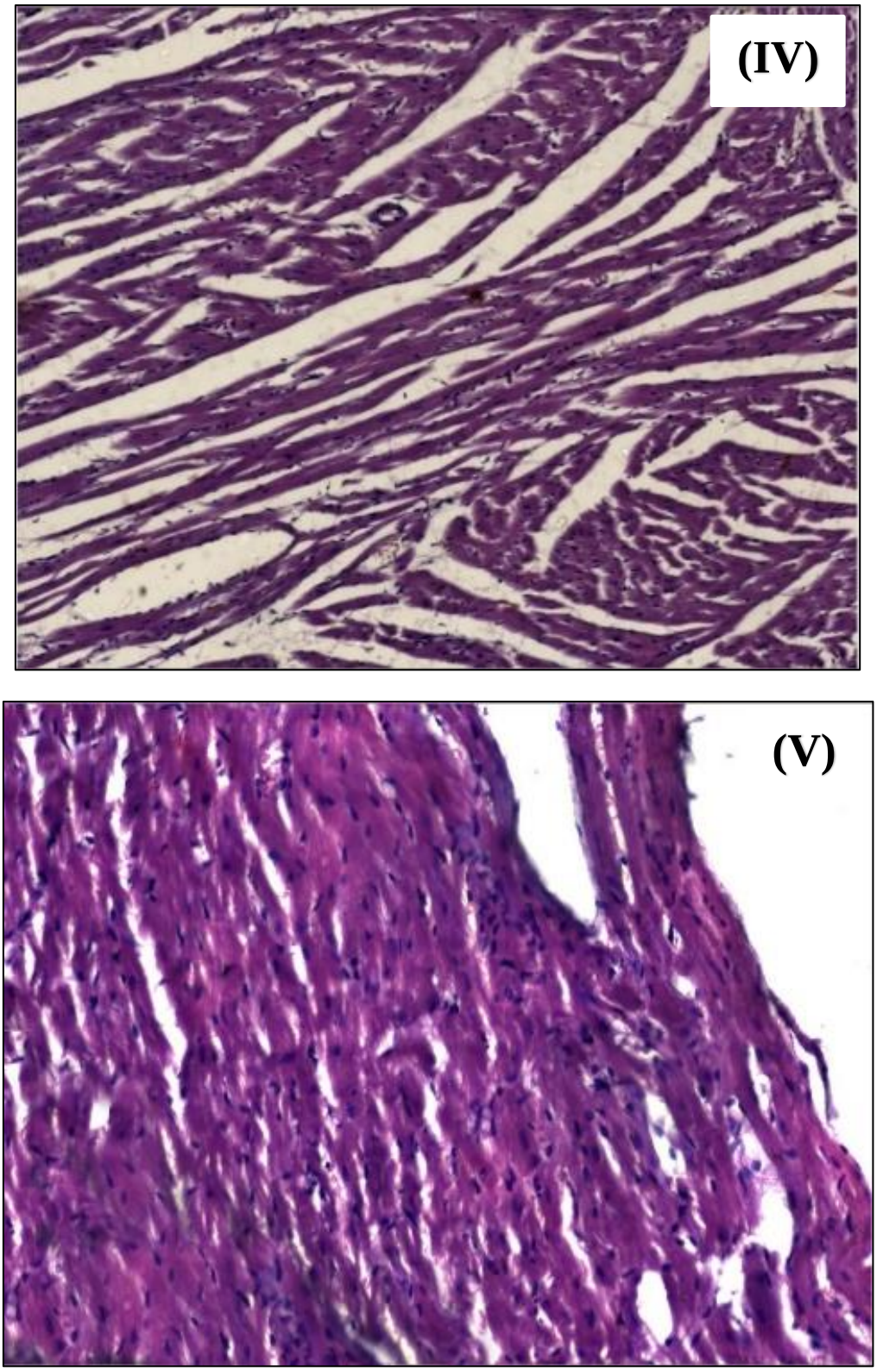
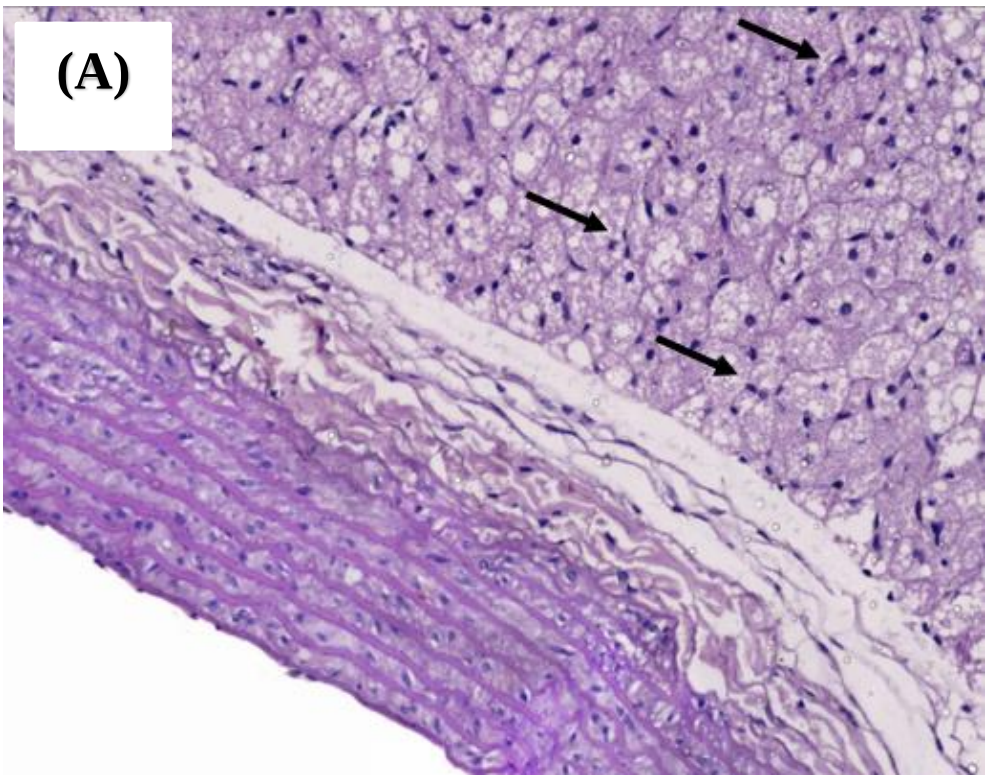
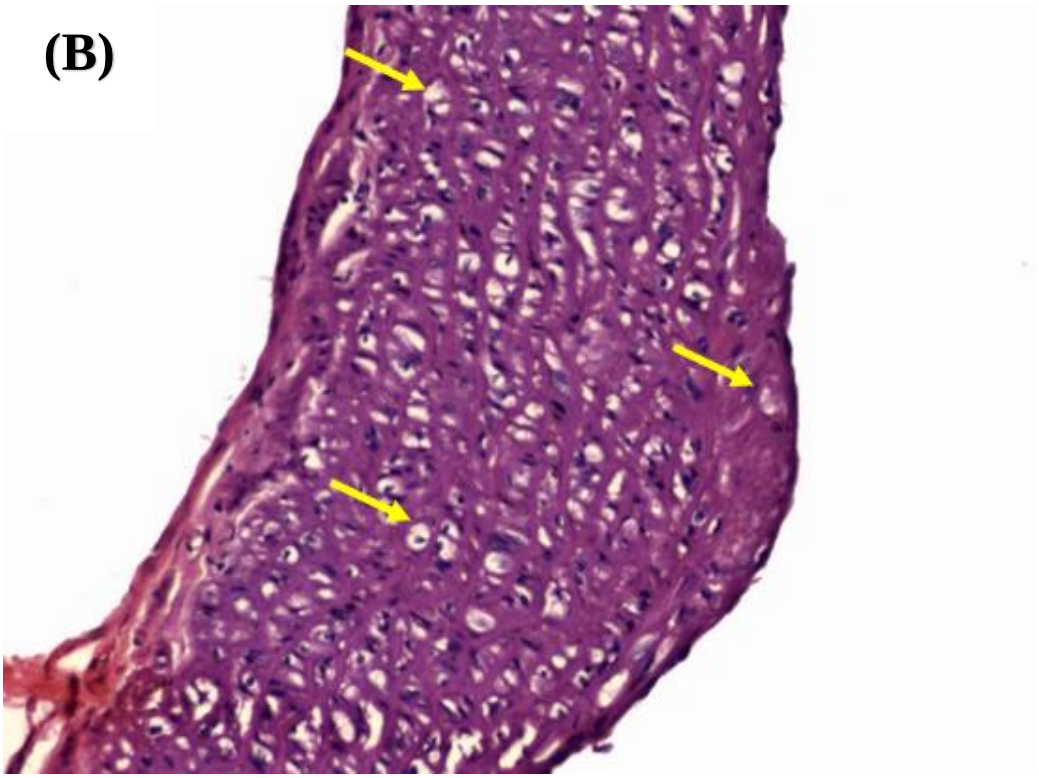
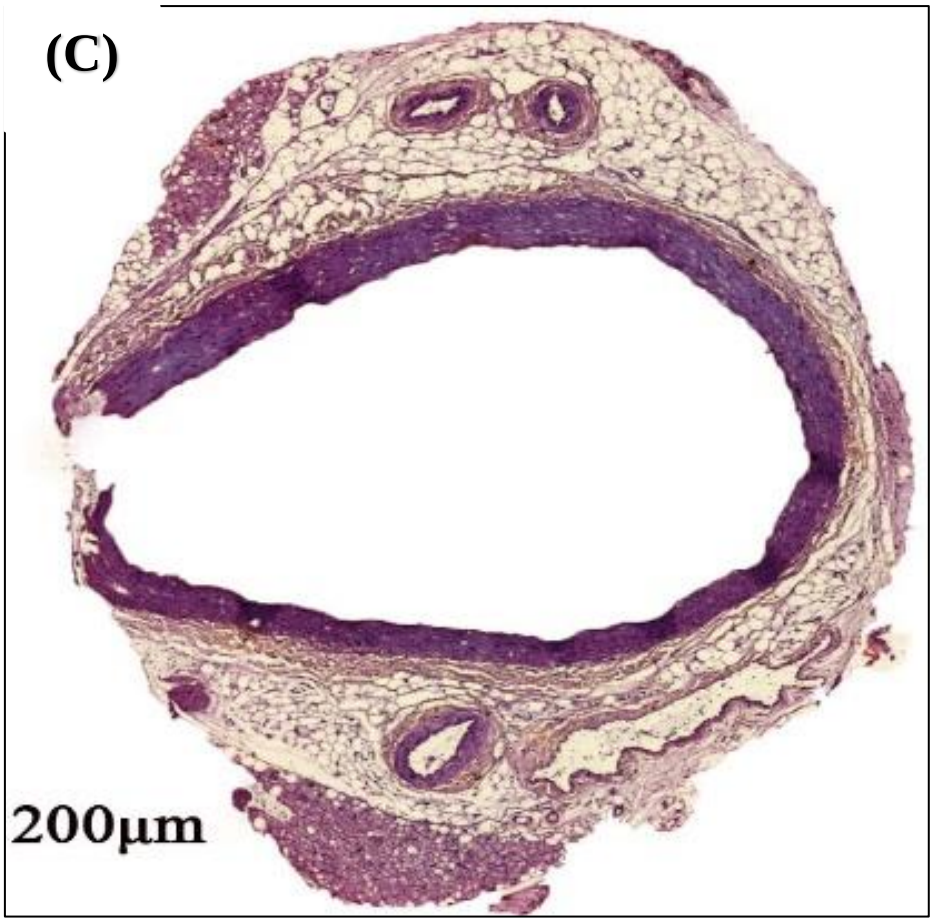
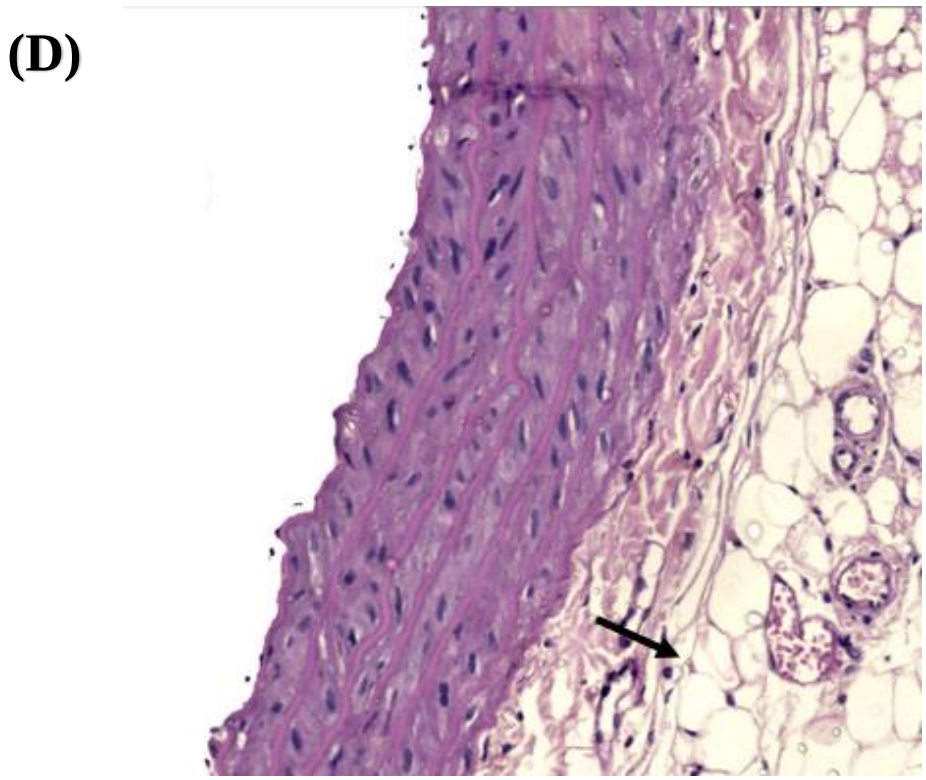



**Figure 9.** Sections of rat heart with hematoxylin & eosin staining **(H&Ex20).**
**(I)** showing normal myocardial fibers **(II)** MetS rat heart showing hemorrhage, necrosis (**red arrows**) and **(III)** collection of inflammatory cells as lymphocytes and histiocytosis (**black arrows**) **(IV)** MetS rat heart treated with rivaroxaban showing no hemorrhage or necrosis with absence of inflammatory cells **(V)** MetS rat heart treated with dabigatran showing decreased inflammatory cells infiltrates.


(A)


(B)


(C)
200µm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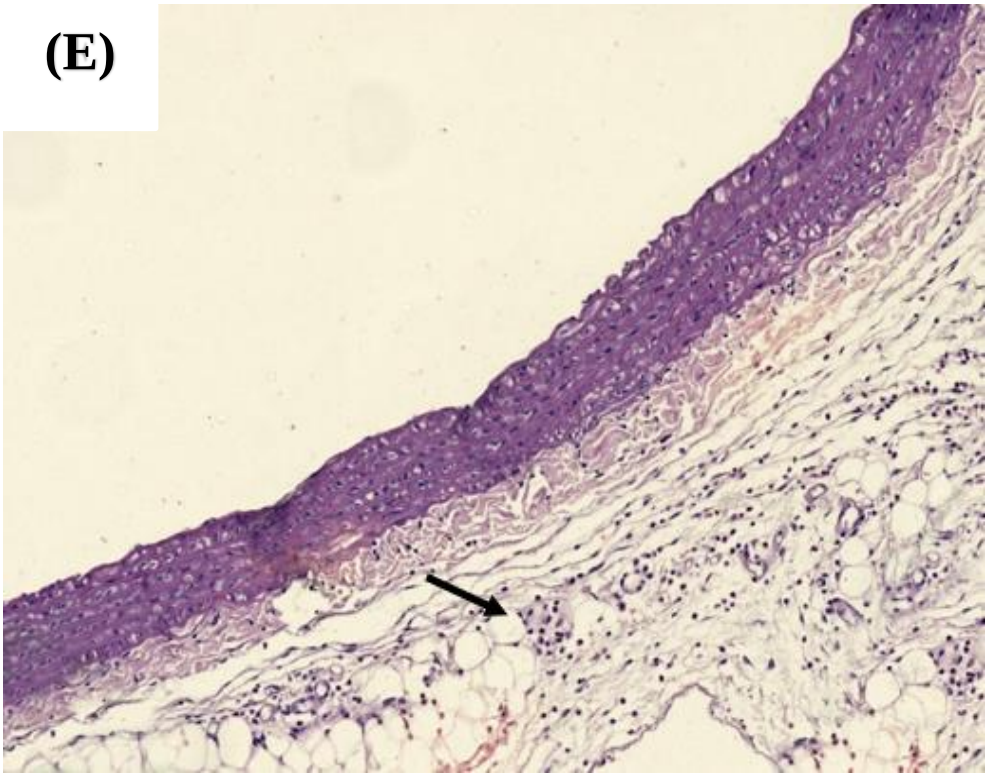
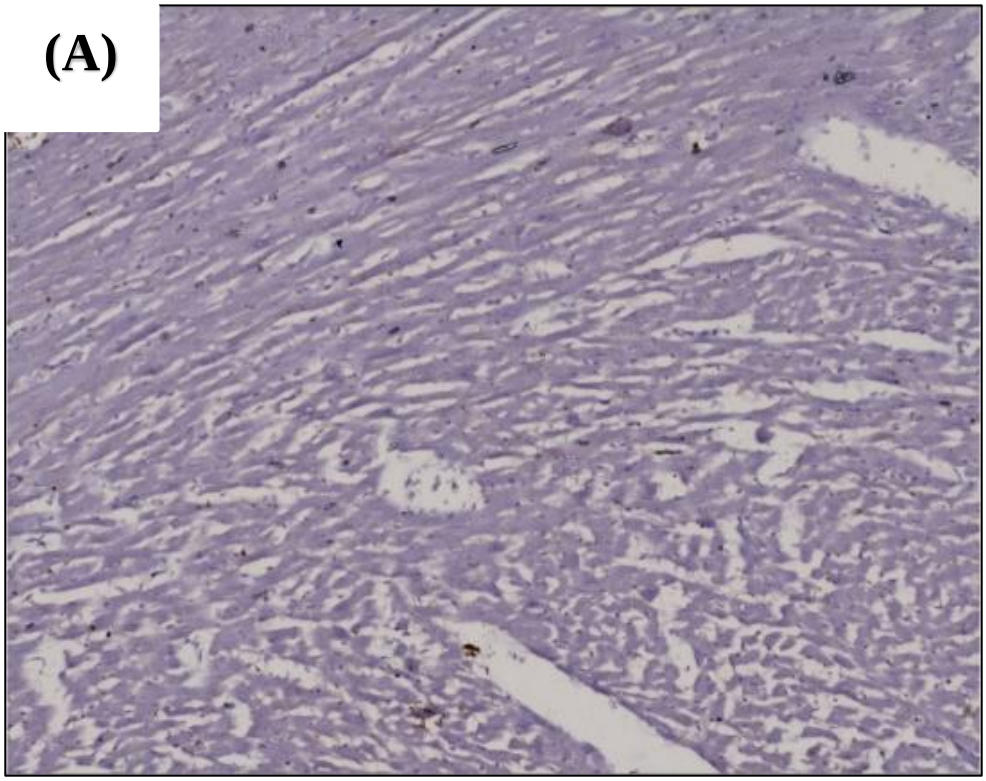
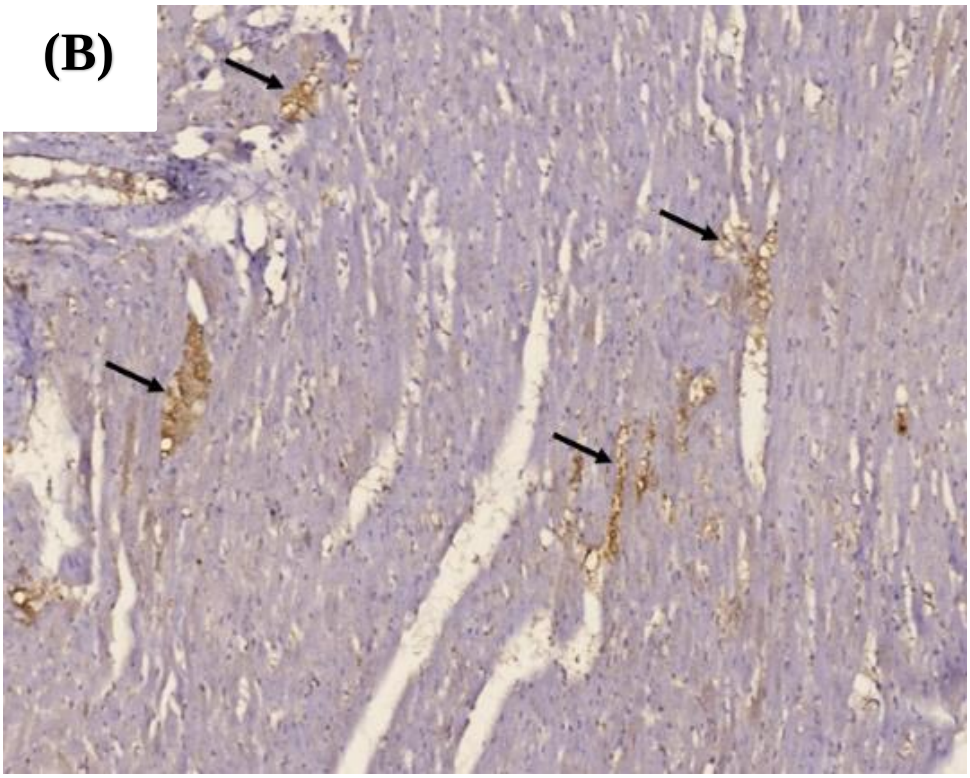



**Figure 10.** Sections of rat aorta hematoxylin & eosin staining **(H&Ex20).**
**(A)** showing normal aorta, **(B)** MetS rat aorta showing perivascular infiltration by inflammatory cells (**black arrows**) and **(C)** infiltration of intima by lymphocytes and histiocytes with mild elevation (**yellow arrows**), **(D)** MetS rat aorta treated with rivaroxaban decreased perivascular and intimal infiltration by inflammatory cells , **(E)** MetS rat aorta treated with dabigatran showing scattered perivascular infiltration with inflammatory cells and decreased intimal infiltration by lymphocytes and foamy cells (**black arrows**).

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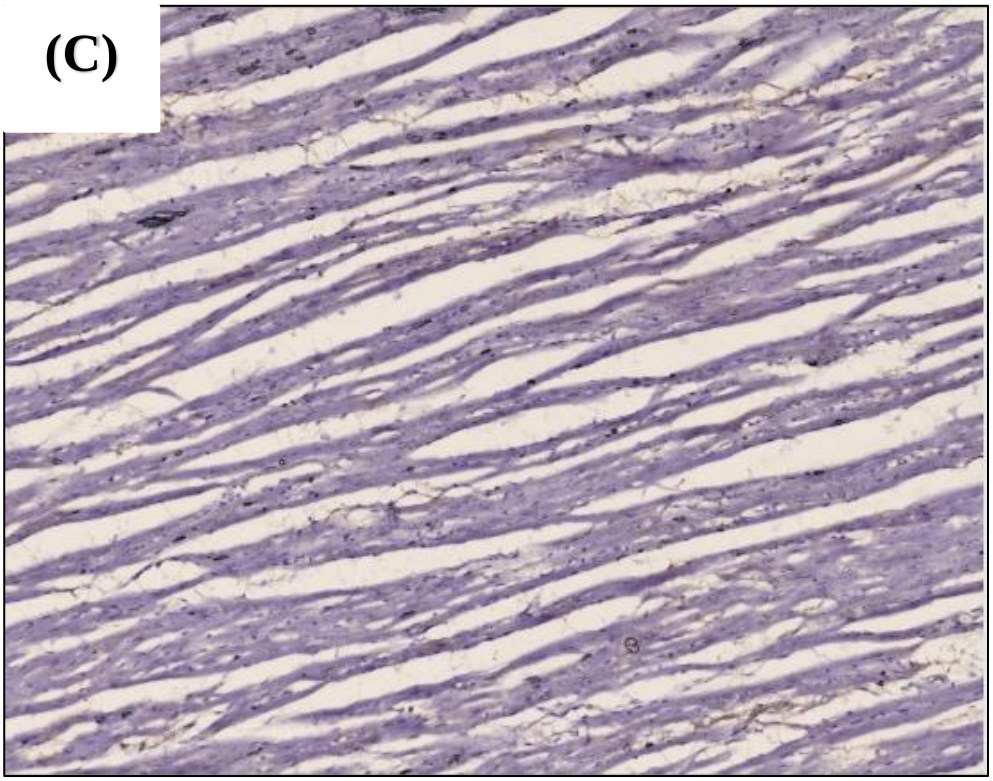
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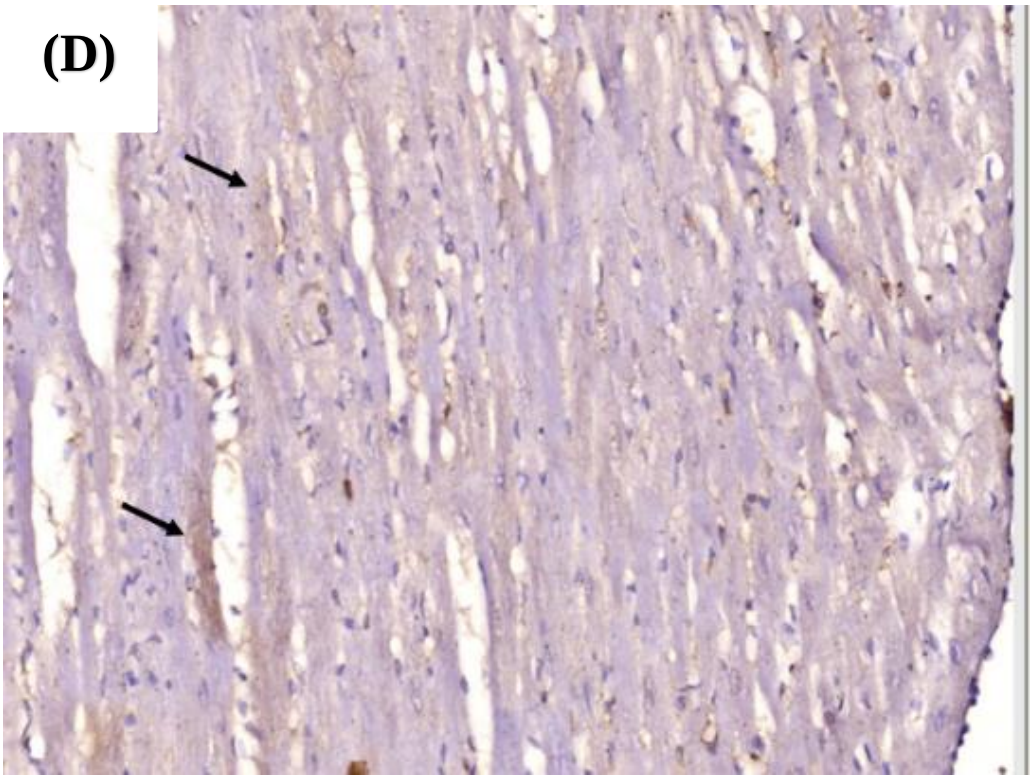
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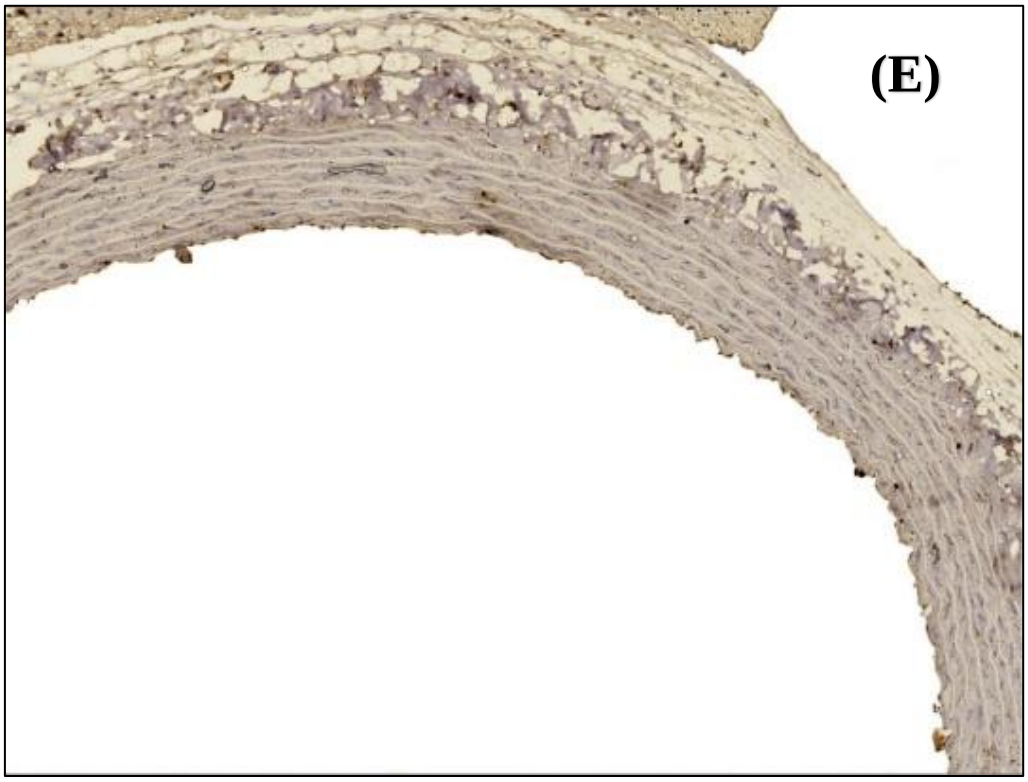
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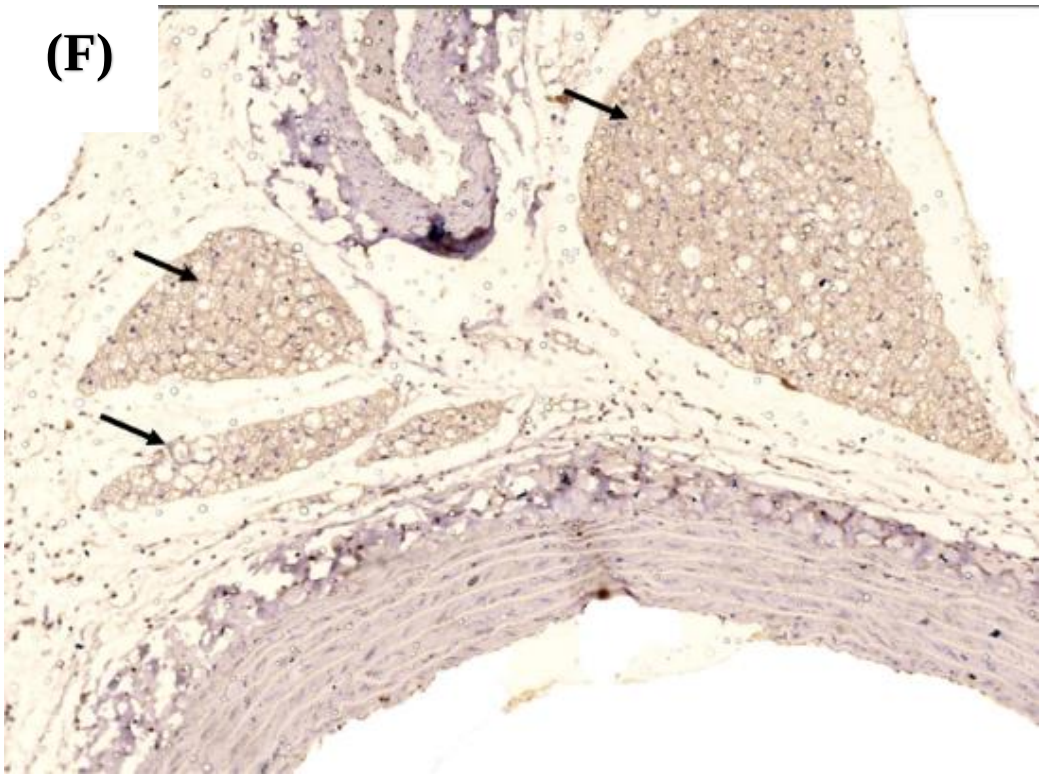
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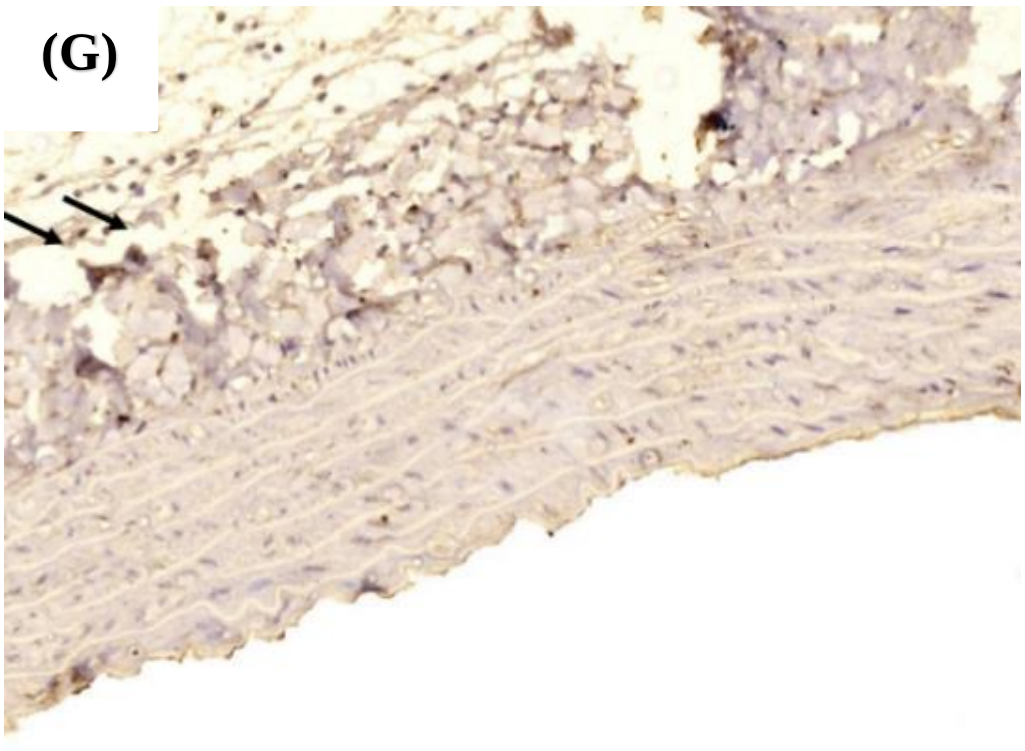
(G)

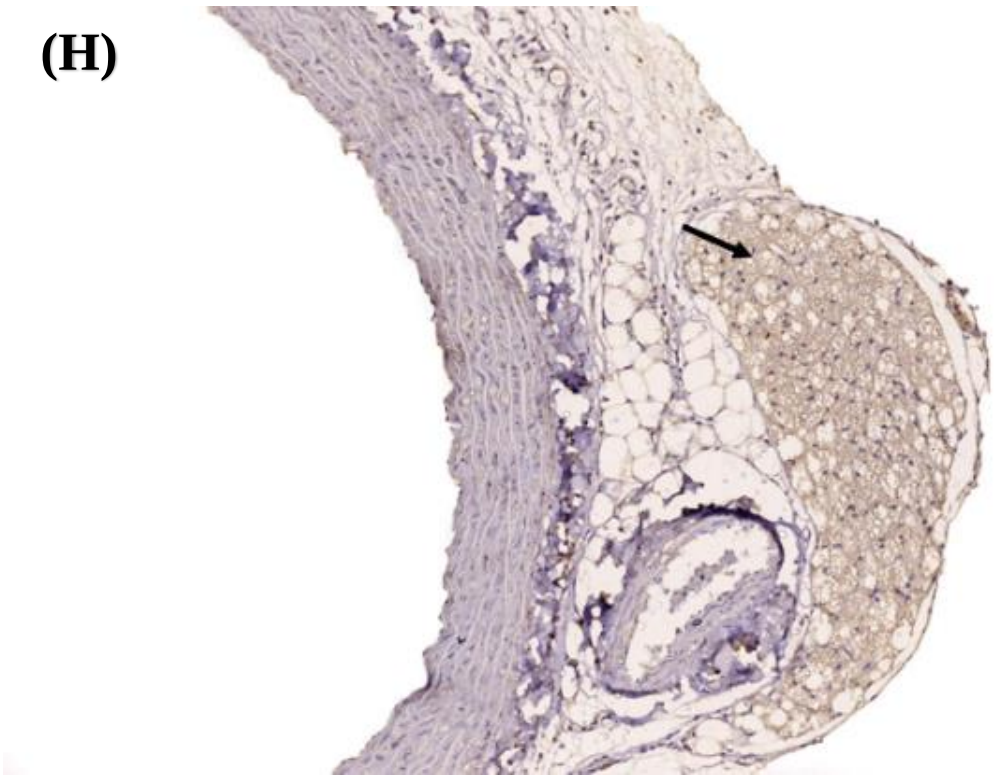


**Figure 11.** Sections of rat heart and aorta with immunohistochemical staining **(TNF-α x 20).**

**(A)** a section of normal cardiac muscle with negative immunostaining for TNF-α. **(B)** a section of MetS rat heart showing positive immunostaining for TNF-α. **(C)** a section of MetS rat heart treated with rivaroxaban showing faint positive immunostaining for TNF-α. **(D)** a section of MetS rat heart treated with dabigatran showing positive cytoplasmic staining for TNF-α detected in some cardiac cells. **(E)** a section of normal aorta with negative immunostaining for TNF-α. **(F)** a section of MetS rat aorta showing positive perivascular immunostaining for TNF-α. **(G)** a section of MetS rat aorta treated with rivaroxaban showing few perivascular positive immunostaining for TNF-α. **(H)** a section of MetS rat aorta treated with dabigatran showing decreased perivascular positive immunostaining for TNF-α.

#### *3.4.2. Fasting blood glucose, insulin level and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) ratio*

MetS rats treated with rivaroxaban or dabigatran showed a statistically significant improvement of the increased fasting blood glucose, insulin and HOMA-IR ratio when compared to the metabolic syndrome group (Table 3 and Figure 5).

#### *3.4.3. Creatine kinase Myocardial Band (CK-MB), Troponin I and Lactic Acid Dehydrogenase (LDH)*

MetS group treated with rivaroxaban or dabigatran showed a significant reduction of the elevated CK-MB, Troponin I and LDH levels when compared to the metabolic syndrome group (Table 3 and Figure 6).

#### *3.4.4. Malondialdehyde (MDA), Glutathione (GSH) and Myeloperoxidase (MPO)*

MetS group treated with rivaroxaban or dabigatran showed a statistically significant reduction of the increased MDA and MPO levels and alleviated the reduction in GSH level when compared to MEtS group. (Table 3 and Figure 7).

#### *3.4.5. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT)*

MetS rats treated with rivaroxaban showed a statistically significant prolongation of the shortened PT as compared to MetS rats, while using dabigatran showed no benefit (Table 3 and Figure 8). MetS treated with dabigatran showed a statistically significant prolongation of the reduced APTT as compared to MetS group and rivaroxaban treated groups (Figure 8).

### *3.5. Histopathological study*

In the present study, specimens of the rat heart and aorta belonging to control and experimental groups were subjected to histological examination. The following results were recorded.

#### *3.5.1. Hematoxylin & Eosin-stained sections*

Histopathological examination sections prepared from a normal rat heart (normal control group) demonstrated normal myocardial fibers with no hemorrhage, congestion, necrosis or inflammatory cells. By induction of metabolic syndrome (MetS group), Sections examined from MetS rat heart revealed hemorrhage, necrosis, and dense chronic inflammatory infiltrate in form of lymphocytes and histiocytes. On the other hand, examination of sections prepared from MetS rat heart treated with rivaroxaban revealed no hemorrhage or necrosis with decreased inflammatory cells as lymphocytes and histiocytes. In addition, a section of MetS rat heart treated with dabigatran revealed also decreased inflammatory cell infiltrate (Figure 9). Sections prepared from the normal rat aorta (normal control group) demonstrated normal architecture of aorta with the absence of foamy cells infiltration in the perivascular layer of the aorta. In contrast, sections examined from MetS rat aorta revealed perivascular infiltration by inflammatory cells and infiltration of intima by lymphocytes and histiocytes with mild elevation. Section of MetS rat aorta treated with rivaroxaban showed decreased perivascular and intimal infiltration by inflammatory cells. Examination of sections prepared from MetS rat aorta treated with dabigatran showed scattered perivascular infiltration with inflammatory cells and decreased intimal infiltration by lymphocytes and foamy cells (Figure 10).

### *3.6. Immunohistochemically sections*

#### *3.6.1. The expression of TNF-α in the tissues of heart*

●***Normal Control group:*** Negative immunohistochemical staining was observed in the tissues of the heart (Figure 11).

●***MetS group:*** Many cells exhibited a positive immunostaining in the tissues of the heart and aorta. The TNF-α staining was observed in cardiac tissues.

●***MetS group treated with rivaroxaban:*** A positive immunostaining was detected in a few cardiac cells.

● ***MetS group treated with dabigatran:*** A positive cytoplasmic staining was detected in some cardiac cells.

#### *3.6.2. The expression of TNF-α in the tissues of aorta*

● ***Normal Control group:*** Negative immunostaining could be detected in the aorta**.**

● ***MetS group:*** Many cells exhibited a positive immunostaining in the tissues of the aorta observed in perivascular tissue of the aorta with a cytoplasmic pattern of expression.

● ***MetS group treated with rivaroxaban:*** A positive immunostaining was detected in few perivascular aortic tissues.

● ***MetS group treated with dabigatran:*** A positive cytoplasmic staining was detected in some cells in perivascular tissue of the aorta.

## 4. Conclusions

Rivaroxaban and dabigatran have potent protective cardiovascular effects on metabolic syndrome and diabetes mellitus, as both drugs showed a remarkable improvement in anthropometric, SBP, and ECG parameters, with a significant refinement of biochemical, histopathological, and immunohistochemically studies' findings when compared to the MetS group.

## References


[1] A.M. Alahdal, A.M. Almohammadi, A.A. Elberry, A.O. Noor, D.M. Almasri, A.A. Bagalagel, M.A. Abdel-Hameed. (2016). Experimental benefit of nebivolol on metabolic changes associated with metabolic syndrome in fructose-fed rats. 5(4) 156-164.

[2] D.P. Cardinali, P.A. Scacchi Bernasconi, R. Reynoso, C.F. Reyes Toso, P. Scacchi. (2013). Melatonin may curtail the metabolic syndrome: studies on initial and fully established fructose-induced metabolic syndrome in rats. International Journal of Molecular Sciences. 14 (2) 2502-2514.

[3] R.K. Suman, I. Ray Mohanty, M.K. Borde, U. Maheshwari, Y.A. Deshmukh. (2016). Development of an experimental model of diabetes co-existing with metabolic syndrome in rats. Advances in Pharmacological and Pharmaceutical Sciences, 2016.

[4] D. Das, N.R. Shruthi, A. Banerjee, G. Jothimani, A.K. Duttaroy, S. Pathak. (2023). Endothelial dysfunction, platelet hyperactivity, hypertension, and the metabolic syndrome: molecular insights and combating strategies. Frontiers in Nutrition. 10.

[5] F. Pomero, M.N.D. Di Minno, L. Fenoglio, M. Gianni, W. Ageno, F. Dentali. (2015). Is diabetes a hypercoagulable state? A critical appraisal. Acta diabetologica. 52 (6) 1007-1016.

[6] K. Urschel, I. Cicha. (2015). TNF-α in the cardiovascular system: from physiology to therapy. International Journal of Interferon, Cytokine and Mediator Research. 9-25.

[7] M.A. Cavender, C.M. Gibson, E. Braunwald, S.D. Wiviott, S.A. Murphy, E. Toda Kato, J.L. Mega. (2015). The effect of rivaroxaban on myocardial infarction in the ATLAS ACS 2-TIMI 51 trial. European Heart Journal: Acute Cardiovascular Care. 4 (5) 468-474.

[8] C. Frost, Y. Song, Y.C. Barrett, J. Wang, J. Pursley, R.A. Boyd, F. LaCreta. (2014). A randomized direct comparison of the pharmacokinetics and pharmacodynamics of apixaban and rivaroxaban. Clinical pharmacology: advances and applications. 179-187.

[9] G.E. Zakynthinos, V. Tsolaki, E. Oikonomou, M. Vavouranakis, G. Siasos, E. Zakynthinos. (2023). Metabolic Syndrome and Atrial Fibrillation: Different Entities or Combined Disorders. Journal of Personalized Medicine. 13 (9) 1323.

[10] M. Brambatti, H. Darius, J. Oldgren, A. Clemens, H.H. Noack, M. Brueckmann, J.S. Healey. (2015). Comparison of dabigatran versus warfarin in diabetic patients with atrial fibrillation: results from the RE-LY trial. International journal of cardiology. 196 127-131.

[11] M.R. Preusch, N. Ieronimakis, E.S. Wijelath, S. Cabbage, J. Ricks, F. Bea, M.E. Rosenfeld. (2015). Dabigatran etexilate retards the initiation and progression of atherosclerotic lesions and inhibits the expression of oncostatin M in apolipoprotein E-deficient mice. Drug design, development and therapy. 5203-5211.

[12] N.P. Kadoglou, M. Stasinopoulou, E. Gkougkoudi, E. Christodoulou, N. Kostomitsopoulos, G. Valsami. (2023). The Complementary Effects of Dabigatran Etexilate and Exercise Training on the Development and Stability of the Atherosclerotic Lesions in Diabetic ApoE Knockout Mice. Pharmaceuticals. 16 (10) 1396.

[13] A. Rahadian, D. Fukuda, H.M. Salim, S. Yagi, K. Kusunose, H. Yamada, M. Sata. (2020). Thrombin inhibition by dabigatran attenuates endothelial dysfunction in diabetic mice. Vascular Pharmacology. 124 106632.

[14] O. Lorenzi, C. Veyrat-Durebex, C.B. Wollheim, P. Villemin, F. Rohner-Jeanrenaud, A.M. Zanchi, U. Vischer. (2010). Evidence against a direct role of klotho in insulin resistance. Pflügers Archiv-European Journal of Physiology. 459 465-473.

[15] A.M. Gajda, M.A. Pellizzon, M.R. Ricci, E.A. Ulman. (2007). Diet-induced metabolic syndrome in rodent models. Animal Lab News. 74 775-793.

[16] Y. Nagai, A. Ichihara, D. Nakano, S. Kimura, N. Pelisch, Y. Fujisawa, A. Nishiyama. (2009). Possible contribution of the non-proteolytic activation of prorenin to the development of insulin resistance in fructose-fed rats. Experimental Physiology. 94 (9) 1016-1023.

[17] F.D. Liu, R. Zhao, X.Y. Feng, Y.H. Shi, Y.L. Wu, X.L. Shen, J.R. Liu. (2018). Rivaroxaban does not influence hemorrhagic transformation in a diabetes ischemic stroke and endovascular thrombectomy model. Scientific reports. 8 (1) 7408.

[18] S. Yazici, O. Karahan, M.K. Oral, Z. Bayramoğlu, M. Unal, B. Caynak, E. Sagbas. (2016). Comparison of renoprotective effect of dabigatran with low-molecular-weight heparin. Clinical and Applied Thrombosis/Hemostasis. 22 (4) 361-365.

[19] N. Matsuura, C. Asano, K. Nagasawa, S. Ito, Y. Sano, Y. Minagawa, K. Nagata. (2015). Effects of pioglitazone on cardiac and adipose tissue pathology in rats with metabolic syndrome. International journal of cardiology. 179 360-369.

[20] H. Van Herck, V. Baumans, C.J.W.M. Brandt, A.P.M. Hesp, J.H. Sturkenboom, H.A. Van Lith, A.C. Beynen. (1998). Orbital sinus blood sampling in rats as performed by different animal technicians: the influence of technique and expertise. Laboratory Animals. 32 (4) 377-386.

[21] Y.B. Wang, Z.M. Ge, W.Q. Kang, Z.X. Lian, J. Yao, C.Y. Zhou. (2015). Rutin alleviates diabetic cardiomyopathy in a rat model of type 2 diabetes. Experimental and therapeutic medicine. 9 (2) 451-455.

[22] S.K. Banerjee, A.K. Dinda, S.C. Manchanda, S.K. Maulik. (2002). Chronic garlic administration protects rat heart against oxidative stress induced by ischemic reperfusion injury. BMC pharmacology. 2 1-9.

[23] J.P. Bantle. (2009). Dietary fructose and metabolic syndrome and diabetes. The Journal of nutrition. 139 (6) 1263S-1268S.

[24] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner. (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. diabetologia. 28 412-419.

[25] H. Soufy, T.S. Lin, S. Das, Z. Zakaria. (2012). Histological changes in the heart and the proximal aorta in experimental diabetic rats fed with Piper sarmentsoum. African Journal of Traditional, Complementary and Alternative Medicines. 9 (3) 396-404.

[26] A.N.N.E. Manktelow, A.A. Meyer. (1986). Lack of correlation between decreased chemotaxis and susceptibility to infection in burned rats. The Journal of Trauma. 26 (2) 143-148.

[27] K. Yagl. (1984). Assay for blood plasma and serum peroxides. Methods Enzymol. 105 28-31.

[28] L. Tappy, K.A. Lê. (2010). Metabolic effects of fructose and the worldwide increase in obesity. Physiological reviews.

[29] Y. Pan, L.D. Kong. (2018). High fructose diet-induced metabolic syndrome: pathophysiological mechanism and treatment by traditional Chinese medicine. Pharmacological research. 130 438-450.

[30] S. Softic, K.L. Stanhope, J. Boucher, S. Divanovic, M.A. Lanaspa, R.J. Johnson, C.R. Kahn. (2020). Fructose and hepatic insulin resistance. Critical reviews in clinical laboratory sciences. 57 (5) 308-322.

[31] A.K. Kopec, S.R. Abrahams, S. Thornton, J.S. Palumbo, E.S. Mullins, S. Divanovic, M.J. Flick. (2017). Thrombin promotes diet-induced obesity through fibrin-driven inflammation. The Journal of clinical investigation. 127 (8) 3152-3166.

[32] M.J. Flick, C.M. LaJeunesse, K.E. Talmage, D.P. Witte, J.S. Palumbo, M.D. Pinkerton, J.L. Degen. (2007). Fibrin (ogen) exacerbates inflammatory joint disease through a mechanism linked to the integrin α M β 2 binding motif. The Journal of clinical investigation. 117 (11) 3224-3235.

[33] S.H. Park, M.A. Farooq, S. Gaertner, C. Bruckert, A.W. Qureshi, H.H. Lee, V.B. Schini-Kerth. (2020). Empagliflozin improved systolic blood pressure, endothelial dysfunction and heart remodeling in the metabolic syndrome ZSF1 rat. Cardiovascular diabetology. 19 1-14.

[34] E.A. Zayed, A.A. AinShoka, K.A. El Shazly, H.A. Abd El Latif. (2018). Improvement of insulin resistance via increase of GLUT4 and PPARγ in metabolic syndrome-induced rats treated with omega-3 fatty acid or l-carnitine. Journal of biochemical and molecular toxicology. 32 (11) e22218.

[35] A. Ring, K. Rathgen, J. Stangier, P. Reilly, A. Clemens, J. Friedman. (2013). Dabigatran does not prolong the QT interval with supratherapeutic exposure: a thorough QT study in healthy subjects. Clinical Drug Investigation. 33 333-342.

[36] D. Kubitza, W. Mueck, M. Becka. (2008). Randomized, double-blind, crossover study to investigate the effect of rivaroxaban on QT-interval prolongation. Drug safety. 31 67-77.

[37] S. Namba, M. Yamaoka-Tojo, R. Kakizaki, T. Nemoto, K. Fujiyoshi, T. Hashikata, J. Ako. (2017). Effects on bone metabolism markers and arterial stiffness by switching to rivaroxaban from warfarin in patients with atrial fibrillation. Heart and vessels. 32 977-982.

[38] V. Barrios, C. Escobar. (2013). Can dabigatran improve blood pressure control?. Future Cardiology. 9 (3) 321-323.

[39] A. Kij, A. Bar, K. Przyborowski, B. Proniewski, L. Mateuszuk, A. Jasztal, S. Chlopicki. (2021). Thrombin inhibition prevents endothelial dysfunction and reverses 20-HETE overproduction without affecting blood pressure in angiotensin II-induced hypertension in mice. International Journal of Molecular Sciences. 22 (16) 8664.

[40] K.M. Ware, J.C. Vance, N. Muni, L.A. Hebert, A.A. Satoskar, G. Nadasdy, S.V. Brodsky. (2015). Oral warfarin and the thrombin inhibitor dabigatran increase blood pressure in rats: hidden danger of anticoagulants?. American journal of hypertension. 28 (2) 182-189.

[41] D. Kubitza, M. Becka, S. Schwers, B. Voith. (2013). Investigation of pharmacodynamic and pharmacokinetic interactions between rivaroxaban and enoxaparin in healthy male subjects. Clinical Pharmacology in Drug Development. 2 (3) 270-277.

[42] X. Lou, Z. Yu, X. Yang, J. Chen. (2019). Protective effect of rivaroxaban on arteriosclerosis obliterans in rats through modulation of the toll-like receptor 4/NF-κB signaling pathway. Experimental and therapeutic medicine. 18 (3) 1619-1626.

[43] F. Imam, N.O. Al-Harbi, M.R. Khan, W. Qamar, M. Alharbi, A.A. Alshamrani, K.S. Alharbi. (2020). Protective effect of RIVA against sunitinib-induced cardiotoxicity by inhibiting oxidative stress-mediated inflammation: probable role of TGF-β and Smad signaling. Cardiovascular Toxicology. 20 281-290.

[44] S.P. Grover, T. Coughlin, S.M. Fleifil, J.J. Posma, H.H. Spronk, S. Heitmeier, N. Mackman. (2022). Effect of combining aspirin and rivaroxaban on atherosclerosis in mice. Atherosclerosis. 345 7-14.

[45] J.J. Posthuma, J.J. Posma, R. van Oerle, P. Leenders, R.H. van Gorp, A.M. Jaminon, H.M. Spronk. (2019). Targeting coagulation factor Xa promotes regression of advanced atherosclerosis in apolipoprotein-E deficient mice. Scientific Reports. 9 (1) 3909.

[46] Q. Zhou, F. Bea, M. Preusch, H. Wang, B. Isermann, K. Shahzad, E. Blessing. (2011). Evaluation of plaque stability of advanced atherosclerotic lesions in apo E-deficient mice after treatment with the oral factor Xa inhibitor rivaroxaban. Mediators of inflammation, 2011.

[47] N.P. Kadoglou, P. Moustardas, M. Katsimpoulas, A. Kapelouzou, N. Kostomitsopoulos, K. Schafer, C.D. Liapis. (2012). The beneficial effects of a direct thrombin inhibitor, dabigatran etexilate, on the development and stability of atherosclerotic lesions in apolipoprotein E-deficient mice: dabigatran etexilate and atherosclerosis. Cardiovascular drugs and therapy. 26 367-374.

[48] P. Joseph, G. Pare, L. Wallentin, S. Connolly, S. Yusuf, J. Wang, J. Oldgren. (2016). Dabigatran etexilate and reduction in serum apolipoprotein B. Heart. 102 (1) 57-62.

[49] H. ten Cate. (2016). Dabigatran and apolipoprotein B. Heart. 102 (1) 5-6.

[50] A. Scridon, A. Mărginean, A. Huțanu, L. Chinezu, D. Gheban, M. Perian, D. Dobreanu. (2019). Vascular protease-activated receptor 4 upregulation, increased platelet aggregation, and coronary lipid deposits induced by long-term dabigatran administration–results from a diabetes animal model. Journal of Thrombosis and Haemostasis. 17 (3) 538-550.

[51] S. Avci, H. Gungor, A.S. Kumru, M. Sahin, A. Gezer, U. Gok, M. Avcil. (2021). Effects of apixaban, rivaroxaban, dabigatran and enoxaparin on histopathology and laboratory parameters in Achilles tendon injury: An in vivo study. Saudi Journal of Medicine & Medical Sciences. 9 (3) 205.

[52] M. Shani, D. Comaneshter, A. Lustman. (2021). Adherence to Oral Anticoagulant Medications. The Israel Medical Association Journal: IMAJ. 23 (9) 580-583.

[53] C.M. Gibson, A.K. Chakrabarti, J. Mega, C. Bode, J.P. Bassand, F.W. Verheugt, ATLAS-ACS 2 TIMI 51 Investigators. (2013). Reduction of stent thrombosis in patients with acute coronary syndromes treated with rivaroxaban in ATLAS-ACS 2 TIMI 51. Journal of the American College of Cardiology. 62 (4) 286-290.

[54] Y. Ma, Y. Zhang, C. Qiu, C. He, T. He, S. Shi, Z. Liu. (2021). Rivaroxaban suppresses atherosclerosis by inhibiting FXa-induced macrophage M1 polarization-mediated phenotypic conversion of vascular smooth muscle cells. Frontiers in cardiovascular medicine. 8 739212.

[55] A. Amin, A.B. Garcia Reeves, X. Li, A. Dhamane, X. Luo, M. Di Fusco, A. Keshishian. (2019). Effectiveness and safety of oral anticoagulants in older adults with non-valvular atrial fibrillation and heart failure. PLoS One. 14 (3) e0213614.

[56] T.G. Thor, V.T.G. Chuang, K.H. Lam. (2019). Dabigatran as the Anticoagulant of Choice for Treating Acute Myocardial infarction in a Patient with Ectatic Coronary Artery and Thrombus. ASEAN Heart Journal. 26 4.

[57] I.O. Lee, M.T. Kratz, S.H. Schirmer, M. Baumhäkel, M. Böhm. (2012). The effects of direct thrombin inhibition with dabigatran on plaque formation and endothelial function in apolipoprotein E-deficient mice. Journal of Pharmacology and Experimental Therapeutics. 343 (2) 253-257.

[58] J.B. Kim, H.J. Joung, J.M. Lee, J.S. Woo, W.S. Kim, K.S. Kim, W. Kim. (2016). Evaluation of the vascular protective effects of new oral anticoagulants in high-risk patients with atrial fibrillation (PREFER-AF): study protocol for a randomized controlled trial. Trials. 17 (1) 1-5.

[59] K. Song, Y. Wang, J. Sheng, C. Ma, H. Li. (2018). Effects of dabigatran regulates no-reflow phenomenon in acute myocardial infarction mice through anti-inflammatory and anti-oxidative activities and connective tissue growth factor expression. Molecular Medicine Reports. 17 (1) 580-585.

[60] J. Douxfils, F. Buckinx, F. Mullier, V. Minet, V. Rabenda, J.Y. Reginster, J.M. Dogné. (2014). Dabigatran etexilate and risk of myocardial infarction, other cardiovascular events, major bleeding, and all-cause mortality: a systematic review and meta-analysis of randomized controlled trials. Journal of the American Heart Association. 3 (3) e000515.

[61] B.A. Steinberg, P. Shrader, L. Thomas, J. Ansell, G.C. Fonarow, B.J. Gersh, ORBIT-AF Investigators and Patients. (2016). Off-label dosing of non-vitamin K antagonist oral anticoagulants and adverse outcomes: the ORBIT-AF II registry. Journal of the American College of Cardiology. 68 (24) 2597-2604.

[62] R. Kerkela, K.C. Woulfe, J.B. Durand, R. Vagnozzi, D. Kramer, T.F. Chu, T. Force. (2009). Sunitinib-induced cardiotoxicity is mediated by off-target inhibition of AMP-activated protein kinase. Clinical and translational science. 2 (1) 15-25.

[63] T. Hara, D. Fukuda, K. Tanaka, Y. Higashikuni, Y. Hirata, S. Nishimoto, M. Sata. (2015). Rivaroxaban, a novel oral anticoagulant, attenuates atherosclerotic plaque progression and destabilization in ApoE-deficient mice. Atherosclerosis. 242 (2) 639-646.

[64] P. Ellinghaus, E. Perzborn, P. Hauenschild, C. Gerdes, S. Heitmeier, M. Visser, V. Laux. (2016). Expression of pro-inflammatory genes in human endothelial cells: Comparison of rivaroxaban and dabigatran. Thrombosis research. 142 44-51.

[65] S. Pingel, V. Tiyerili, J. Mueller, N. Werner, G. Nickenig, C. Mueller. (2014). Experimental research Thrombin inhibition by dabigatran attenuates atherosclerosis in ApoE deficient mice. Archives of Medical Science. 10 (1) 154-160.

[66] S.R. Coughlin. (2000). Thrombin signalling and protease-activated receptors. Nature. 407 (6801) 258-264.

[67] M.M. Samama, G. Contant, T.E. Spiro, E. Perzborn, L. Le Flem, C. Guinet, J.L. Martinoli. (2013). Laboratory assessment of rivaroxaban: a review. Thrombosis journal. 11 (1) 1-7.

[68] A. Hillarp, F. Baghaei, I.F. Blixter, K.M. Gustafsson, L. Stigendal, M. Sten-Linder, T.L. Lindahl. (2011). Effects of the oral, direct factor Xa inhibitor rivaroxaban on commonly used coagulation assays. Journal of Thrombosis and Haemostasis. 9 (1) 133-139.

[69] P. Frączek, M. Krzysztofik, A. Stanisz, A. Undas. (2019). Clinical outcomes and plasma clot permeability and lysability in patients with venous thromboembolism on rivaroxaban: a cohort study. Polskie Archiwum Medycyny Wewnętrznej= Polish Archives of Internal Medicine. 129 (6).

[70] P.C. Wong, E.J. Crain, C..A Watson, B. Xin. (2009). Favorable therapeutic index of the direct factor Xa inhibitors, apixaban and rivaroxaban, compared with the thrombin inhibitor dabigatran in rabbits. Journal of Thrombosis and Haemostasis. 7 (8) 1313-1320.

[71] W. Wienen, J.M. Stassen, H. Priepke, U.J. Ries, N. Hauel. (2007). Antithrombotic and anticoagulant effects of the direct thrombin inhibitor dabigatran, and its oral prodrug, dabigatran etexilate, in a rabbit model of venous thrombosis. Journal of Thrombosis and Haemostasis. 5 (6) 1237-1242.

[72] S. Redondo, M.P. Martínez, M. Ramajo, J. Navarro-Dorado, A. Barez, T. Tejerina. (2011). Pharmacological basis and clinical evidence of dabigatran therapy. Journal of hematology & oncology. 4 (1) 1-7.

[73] T. Baglin, D. Keeling, S. Kitchen. (2012). Effects on routine coagulation screens and assessment of anticoagulant intensity in patients taking oral dabigatran or rivaroxaban: guidance from the British Committee for Standards in Haematology. British journal of haematology. 159 (4) 427-429.

[74] J. Van Ryn, J. Stangier, S. Haertter, K.H. Liesenfeld, W. Wienen, M. Feuring, A. Clemens. (2010). Dabigatran etexilate–a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. Thrombosis and haemostasis. 103 (06) 1116-1127.

[75] T.L. Lindahl, F. Baghaei, I.F. Blixter, K.M. Gustafsson, L. Stigendal, M. Sten-Linder, A. Hillarp. (2011). Effects of the oral, direct thrombin inhibitor dabigatran on five common coagulation assays. Thrombosis and haemostasis. 105 (02) 371-378.

[76] B.T. Samuelson, A. Cuker, D.M. Siegal, M. Crowther, D.A. Garcia. (2017). Laboratory assessment of the anticoagulant activity of direct oral anticoagulants: a systematic review. Chest. 151 (1) 127-138.

[77] H. Imano, R. Kato, S. Tanikawa, F. Yoshimura, A. Nomura, Y. Ijiri, T. Hayashi. (2018). Factor Xa inhibition by rivaroxaban attenuates cardiac remodeling due to intermittent hypoxia. Journal of pharmacological sciences. 137 (3) 274-282.

[78] N.I. Mahmoud, B.A.S. Messiha, A.A. Abo-Saif, M.S. Abdel-Bakky. (2019). Inhibition of activated factor X; a new pathway in ameliorating carbon tetrachloride–induced liver fibrosis in rats. Journal of Biochemical and Molecular Toxicology. 33 (5) e22287.

[79] A. Dong, P. Mueller, F. Yang, L. Yang, A. Morris, S.S. Smyth. (2017). Direct thrombin inhibition with dabigatran attenuates pressure overload-induced cardiac fibrosis and dysfunction in mice. Thrombosis research. 159 58-64.

[80] R.H. van Gorp, I. Dijkgraaf, V. Bröker, M. Bauwens, P. Leenders, D. Jennen, L.J. Schurgers. (2021). Off-target effects of oral anticoagulants–vascular effects of vitamin K antagonist and non-vitamin K antagonist oral anticoagulant dabigatran etexilate. Journal of Thrombosis and Haemostasis. 19 (5) 1348-1363.

[81] S. Durmaz, T. Kurtoğlu, Ö.F. Rahman, C. Tataroğlu, M. Yılmaz, E. Barbarus, M.H. Erkan. (2022). Direct oral anticoagulant agents attenuate temporary aortic occlusion-induced renal oxidative and inflammatory responses in rats. Turkish Journal of Thoracic and Cardiovascular Surgery. 30 (2) 184.